An integrated analysis of gut microbiota and the brain transcriptome reveals host-gut microbiota interactions following traumatic brain injury

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\textbf{ABSTRACT}

\textbf{Objectives:} Recent evidence suggests that there is a link between gut and brain via microbial, immune, endocrine and neural signaling pathways, but the changes of gut-brain axis following brain trauma has not yet been clearly shown. The aim of this study was to reveal the gut microbiota and transcriptomic profile of the cerebral cortex in traumatic brain injury (TBI) mice.

\textbf{Methods:} A controlled cortical impact (CCI) device was used to establish a TBI model. Behavioral testing and histopathological analysis were performed. The gut microbiota was analyzed by 16S rRNA sequencing, and gene expression in the cerebral cortex was detected by whole-transcriptome sequencing (RNA-Seq) 7 days after TBI.

\textbf{Results:} The analysis of 16S rRNA sequencing data indicated that TBI increased the relative abundance of Bifidobacterium. The TBI group showed a disturbance in intestinal flora. RNA-Seq analysis identified 523 differentially expressed genes (481 upregulated and 42 downregulated) in the cerebral cortex of the TBI group compared with the sham group. Cluster analysis revealed 93 immune system process-related genes and 55 inflammatory response-related genes that were differentially expressed.

\textbf{Conclusions:} This manuscript reports pathogenic changes via the gut-brain axis driven by TBI, which confer persistent symptoms and susceptibility to neurodegeneration.

1. Introduction

Traumatic brain injury (TBI), one of the leading causes of mortality and long-term disability, is a serious health burden worldwide (Maas et al., 2008). As a trauma-related injury, TBI occurs when the brain is exposed to a mechanical external force, and the injuries range from mild to moderate to severe (Roozenbeek et al., 2013). The severity of TBI is often monitored according to the clinical features of the patient using the Glasgow Coma Scale (Menon et al., 2015). It is also well accepted that accurate classification of early TBI helps to determine the severity; treatment and outcome (Balestrieri et al., 2004).

In general, TBI is divided into two phases: primary and secondary injury. The primary injury is a result of the initial local impact, while the secondary injury during the postinjury period induces a cascade of pathophysiological events (Roth et al., 2014). The immune response and inflammatory response are important components of secondary TBI and include activation of immune cells and aggregation of inflammatory factors (Jassam et al., 2017). After TBI, the blood–brain barrier (BBB) is damaged, and white blood cells in the peripheral circulation infiltrate the central nervous system. These leukocytes secrete cytokines, cytotoxic proteases, and reactive oxygen species that activate the immune activity of CNS glial cells (Simon et al., 2017). Cytokines and chemokines released by microglia, astrocytes, cerebrovascular endothelial cells and peripheral immune cells are significantly increased after TBI. As innate immune cells in the nervous system, microglia play an important role in the whole neuroinflammatory environment. Regulating the activation state of microglia and changing the inflammatory response to promote nerve repair and regeneration may be a potential strategy for the treatment of TBI (Younger et al., 2019). Our study explored the differences in gene expression, biological processes and molecular functions of the cerebral cortex after TBI by analyzing the brain transcriptome.

Furthermore, TBI can also cause functional and structural damage to the intestine, including disruption of the intestinal barrier, intestinal mucosal permeability, and changes in intestinal flora (Chu et al., 2019). Intestinal microecology is very important for human health. The gut microbiota is diverse and abundant, and the number of bacterial genomes exceeds even the number of human genes (Sender et al., 2016).
The gut–brain axis is a communication system that integrates neural, hormonal and immunological signaling between the gut and the brain and provides the intestinal microbiota and its metabolites with a potential route through which to access the brain (Collins et al., 2012). The gut microbiota has been found to be closely linked to the pathological processes of several neurological diseases, such as Parkinson’s disease (Lin et al., 2019), Alzheimer’s disease (Kowalski and Mulak, 2019), depression and anxiety (Zhu et al., 2018). Treangen et al. previously reported that acute TBI induces a decrease in the abundance of *Lactobacillus gasseri*, *Ruminococcus flavefaciens*, and *Eubacterium ventriosum* and an increase in the abundance of *Eubacterium rectum* and *Marvinbryantia formategixens* (Treangen et al., 2018). Nicholson et al. have shown that TBI profoundly affects the composition of the gut microbiome and that this effect is correlated with the severity of brain injury (Nicholson et al., 2019). However, the specific mechanism is not clear.

In our study, we used a controlled cortical impact (CCI) device to establish a TBI model. The gut microbiota was sequenced by 16S rRNA, and gene expression in the cerebral cortex was assessed by whole-transcriptome sequencing. We aimed to investigate the changes in the intestinal microbiota and gene expression in the cerebral cortex after TBI, and to identify the pathophysiologic mechanism of TBI. This manuscript provides novel applications in exploring the pathologic process, molecular mechanism, and therapeutic intervention of TBI in the future.

2. Materials and methods

2.1. Animals and ethics statement

All of the experimental protocols were approved by the Ethics Committee on Experimental Animals at Zhejiang University and were performed in accordance with the ARRIVE guidelines. Eight-week-old C57BL/6 male mice were purchased from Zhejiang Experimental Animal Center and kept in each cage with a 12-h light/dark cycle. The temperature was 24 ± 2 °C, and the mice had free access to food and water. The beam and rotarod tests were performed every day, and the mice were sacrificed at 1 d, 3 d, and 7 d after injury (n = 3). The sham group was subjected to the same procedures but was not injured (n = 3). In the experiments of gut microbiota and brain transcriptome, there were 5 mice each in the TBI group and sham group, the mice were sacrificed at 7 d after injury.

2.2. CCI model

C57BL/6 mice were anesthetized with 4 % isoflurane, and then the maintenance dose was adjusted to 2 % isoflurane. Later, mice were placed in a stereotactic apparatus to ensure that the brain was stable and secure. The hair was shaved, and the skull was exposed. A 3-mm circle was drawn 2 mm lateral from the midline and 2 mm posterior from bregma. The impact depth was set 2.0 mm. The actuator (a 2-mm-diam.ter, rounded tip) was driven at a velocity of 3 m/s with a dwell time of 200 μs using an impact system (The PinPoint™ PCI3000 Precision Cortical Impactor, Hatteras Instrument Cary, NC, USA).

2.3. Behavioral testing

To identify sensory motor function, beam and rotarod tests (n = 3–9 mice/group) were performed within 7 days postinjury. Animals were tested three times at approximately 30 min intervals, and the average was used. Animals were trained and baseline data were generated one day before TBI.

For the beam test, mice were placed on a suspended narrow wooden beam (20 mm wide, 100 cm long), and the duration it remained on the beam was recorded (the maximum limit was 60 s). For the rotarod test, mice were placed on the rods at constant 5 rpm, then accelerated within 5 min from 5 to 40 rpm and kept at 40 rpm for 5 min. The maximum velocity (rpm) and the latency to fall (min) were recorded.

2.4. Histopathological analysis

All histopathological examinations were performed using standard laboratory procedures. The brain tissues were embedded in paraffin blocks, sliced into 5 μm thick sections and mounted onto glass slides. After hematoxylin–eosin (HE) staining, the slides were observed, and photos were taken using an optical microscope (Olympus BX50, Moticam 2306, Japan). The pathologist who performed the observation and analysis was blinded to the groups.

2.5. RNA preparation, cDNA library construction and sequencing

The mice were sacrificed at 7 days postinjury (n = 5). When removing hippocampus, cerebellum, brainstem, and other tissues, the total RNA from the cerebral cortex was extracted using TRIzol reagent (Invitrogen, CA, USA) following the manufacturer’s procedure, and RNA quality was assessed using a NanoDrop ND-1000 (Wilmington, DE, USA). RNA samples with an RNA integrity number (RIN) > 7.0, OD260/280 > 1.8 and total RNA > 1 μg were sent for RNA sequencing.

The obtained RNA samples were further purified, fragmented into small pieces, reverse-transcribed to create the final cDNA library from cerebral cortex samples of *Mus musculus* according to the manufacturer’s instructions. The average insert size for the final cDNA library was 300 ± 50 bp. Finally, we performed 2 × 150 bp paired-end sequencing (PE150) on an Illumina NovaSeq™ 6000 (LC-Bio Technology CO., Ltd., Hangzhou, China) following the vendor’s recommended protocol.

2.6. RNA sequencing analysis

After removing the reads that contained adaptor contamination and the low-quality and undetermined bases, the mapped reads of each sample were assembled using StringTie (Pertea et al., 2015) with default parameters. Then, all transcriptomes from all samples were merged to reconstruct a comprehensive transcriptome. After the final transcriptome was generated, StringTie and ballgown (Frazee et al., 2015) were used to estimate the expression levels of all transcripts and obtain the expression levels of mRNAs by calculating FPKM (FPKM = [total_exon_fragments/mapped_reads (millions)] × exon_length (kb))). Differentially expressed genes (DEGs) between the TBI and sham groups with a fold change >2 or fold change <0.5 and p value <0.05 were selected by R package edgeR (https://bioconductor.org/packages/release/bioc/html/edgeR.html) or DESeq2 (https://www.bioconductor.org/packages/release/bioc/html/DESeq2.html), and then Gene Ontology (GO) enrichment (Young et al., 2010) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment (Kanehisa et al., 2008) analyses were performed.

2.7. 16S rDNA sequencing and gut microbiota analysis

DNA was extracted from fecal samples of the severe injury group at 7 days postinjury using the E.Z.N.A.® Stool DNA Kit (D4015, Omega, Inc., USA) according to the manufacturer’s instructions. The reagent, which was designed to recover DNA from trace amounts of sample, has been shown to be effective for the preparation of DNA of most bacteria. Nuclease-free water was used for the blank. The total DNA was eluted in 50 μL of elution buffer and stored at −80 °C.

PCR amplification was performed and confirmed with 2 % agarose gel electrophoresis. The PCR products were then purified, quantified, and prepared for sequencing. The size and quantity of the amplicon library were assessed on an Agilent 2100 Bioanalyzer (Agilent, USA) and with the Library Quantification Kit for Illumina (Kapa Biosciences, Woburn, MA, USA), respectively, on the NovaSeq PE250 platform (LC-BioTechnologies, Co., Ltd., Hangzhou).

Alpha diversity and beta diversity were calculated by randomly
normalizing to the same sequences to compare the different bacterial community structures among different experimental groups. Alpha diversity was used to analyze the complexity of species diversity for a sample with 5 indexes, the Chao1, observed species, Good’s coverage, Shannon, and Simpson indexes, which were calculated with QIIME2 (Caporaso et al., 2010). According to the principal component analysis (PCA), beta diversity considered the difference of bacterial community composition between TBI and sham groups. The first 30 species with the highest abundance in each taxonomic hierarchy (phylum, class, order, family, genus, species) were selected to draw a cumulative cylindrical map of the relative abundance of species generated by a taxonomic tree. Linear discriminant analysis effect size (LEfSe) software was used to compare the species differences among groups. Linear discriminant analysis (LDA) was used to identify the different intestinal bacteria among the groups (LDA score >3). Diagrams were generated using the R package (v3.5.2).

2.8. Statistical analysis

All data are presented as the mean ± SEM. One-way variance (ANOVA) with LSD tests was applied to evaluate the statistical significance between the TBI and the sham group. Pearson correlation analysis between altered fecal microbiota stains and differentially expressed genes was conducted, produce the correlation coefficient (r) and p value. The repeated measures analysis of behavioral tests after TBI was performed. For all the results, *P < 0.05; **P < 0.01; ***P < 0.001.

3. Results

3.1. Effects of TBI on brain morphology and behavioral performance

Fig. 1 shows the behavioral test results and histological changes in perfused and fixed mouse brains from mice in the sham and TBI groups. Seven days after TBI, no weight-loss, malnutrition, weakness, and death were observed in the experimental mice. In the beam test (Fig. 1A), significant change was found at 1 day post-TBI, with an overall decline of motor function at other time points but no statistical significance. The rotate test showed a significant decrease in motor function 1–6 days after TBI (p < 0.05), accompanied by a gradual recovery (Fig. 1B).

Photomicrographs (original magnification, 1× and 400×; HE staining) show brain biopsies after trauma, demonstrating a histological feature in the cortex (Fig. 1C & D). Consistent with the behavioral tests, the results also showed that in the cerebral cortex of TBI group, there was a recovery of the pathological lesions. However, the hippocampus can hardly be distinguished in TBI model.

3.2. Effects of TBI on gut microbiota

The structure and composition of gut microbiota communities at the phylum, class, order, family, genus and species levels (top thirty) are shown in Fig. 3. The top three relative abundances of bacteria at the phylum level were Firmicutes, Bacteroidetes and Proteobacteria, which accounted for more than 83 % of the total. The top three relative abundances of bacteria at the class level were Bacteroidia, Bacilli, and Clostridia, which accounted for 66.76 % to 92.31 % of the total. Consistently, the top three relative abundances of bacteria at the order level were Bacteroidales, Lactobacillales, and Clostridiales. At the family, genus and species levels, the classification of bacteria tended to vary.

There was no significant change in the total number of observed species (Fig. 2A). The comparison of alpha (α) and beta (β) diversity of gut microbiota between the TBI and sham groups is also shown in Fig. 2. The Shannon, Simpson, Chao1, and goods_coverage indexes were used for the comparison of alpha (α) and beta (β) diversity of gut microbiota, and none of these changed significantly.

However, LeFSe analysis identified some differentially expressed strains among different groups (Fig. 4). Thirty-five differentially expressed strains (LDA score >3 or < −3) were obtained after TBI. The top 20 included p_actinobacteria, o_bifidobacteriales, f_bifidobacteriaceae, g_bifidobacterium, c_actinobacteria, s_lactobacillus_crispatus, s_streptococcus_equinus, g_streptococcus, f_streptococcaceae, s_bifidobacterium_unclassified, s_bididobacterium_pseudolongum, s_parasutterella_unclassified, g_parasutterella, s_lactobacillus_sp_bl303, f_atopobiaceae, g_olsenella, s_olsenella_sp_f0206, s_uncultur ed_bifidobacterium_sp, o_coriobacteriales, and c_coriobacteriia.

Fig. 1. TBI mice show evidence of motor function impairment at 7 days postinjury: (A) Beam test; (B) Rotarod test; (C&D) H&E staining of the mouse brain following TBI and sham procedures: 1× and 400×. *p < 0.05, **p < 0.01, ***p < 0.001.
3.3. Effects of TBI on gene expression in the brain cortex

A total of 523 genes were significantly changed between the TBI groups and sham group in the ipsilateral hemisphere, including 481 upregulated genes and 42 downregulated genes (Fig. 5a). Similarly, 456 upregulated genes and 26 downregulated genes were identified between the ipsilateral and contralateral hemispheres in TBI mice.

Gene ontology of the significantly upregulated genes 7 days post-injury revealed enrichment of 20 GO terms (Fig. 5b). Sixteen biological process terms were enriched, including immune system process, inflammatory response, response to bacterium, positive regulation of interleukin-6 production, immune response, cellular response to lipopolysaccharide, defense response to virus, innate immune response, chemotaxis, cellular response to interferon-gamma, cellular response to interferon-beta, response to virus, neutrophil chemotaxis, microglial cell activation, negative regulation of viral genome replication, and Toll-like receptor signaling pathway. Three cell component terms were enriched: cell surface, extracellular space, and extracellular region. One molecular function term, peptide antigen binding, was enriched.

The top 10 terms from the KEGG database that were enriched included phagosome, leishmaniasis, Staphylococcus aureus infection, tuberculosis, Epstein-Barr virus infection, NOD-like receptor signaling pathway, chemokine signaling pathway, Toll-like receptor signaling pathway, pertussis, and osteoclast differentiation (Fig. 5c).

3.4. Inflammatory response in the cerebral cortex tissue of TBI mice

Of the differentially expressed genes in the inflammatory response pathway, 54 genes had significantly increased expression levels, and 1 gene had decreased expression levels. Thirteen genes were enriched in the Toll-like receptor signaling pathway (Fig. 6): CXCL9 (FC = 2870.05, p < 0.01), TICAM2 (FC = 3.52, p < 0.01), PIK3CG (2.11, p < 0.01), TLR1 (FC = 5.38, p < 0.01), CXCL10 (FC = 69.38, p < 0.01), CCL5 (FC = 99.38, p < 0.01), TLR9 (FC = 2.14, p < 0.01), CCL3 (FC = 21.84, p < 0.01), TLR7 (FC = 2.97, p < 0.01), TLR6 (FC = 2.45, p < 0.01), CD14 (FC = 3.52, p < 0.01), TLR4 (FC = 2.08, p < 0.01), TLR2 (FC = 6.84, p < 0.01), and TLR13 (FC = 3.05, p < 0.01).

Fifteen genes were enriched in the chemokine signaling pathway (Fig. 7): CCR1 (FC = 2.25, p < 0.01), LYN (FC = 2.55, p < 0.01), CCL12 (FC = 8.41, p < 0.01), CXCL9 (FC = 2870.05, p < 0.01), NCF1 (FC =
2.77, \( p < 0.01 \), PIK3CG (FC = 2.11, \( p < 0.01 \)), CXCL5 (FC = 10.85, \( p < 0.01 \)), CXCL1 (FC = 0.39, \( p < 0.01 \)), CXCL10 (FC = 69.38, \( p < 0.01 \)), HCK (FC = 2.39, \( p < 0.01 \)), CCL9 (FC = 21.84, \( p < 0.01 \)), CCL6 (FC = 2.10, \( p < 0.01 \)), CCL5 (FC = 99, \( p < 0.01 \)), CCL5).

3.5. Integrated analysis of gut microbiota and the brain transcriptome in TBI mice

Thirty-five altered fecal microbiota (LDA score >3 or <−3) and 523 DEGs (fold change >2 or <0.5) were selected as candidate biomarkers. To explore the relationship between gut microbiota and the brain inflammation in TBI mice, we found that, Actinobacteria, e_Acinetobacter, o_Bifidobacteriales, f_Bifidobacteriaceae, g_Bifidobacterium, and s_uncultured_Bifidobacterium_sp. were negatively associated with Adgre1, Haver2, Eif2ak2, Sting1, Csf1r, Tir13, Apobec3, Cd300c2, and H2-Q7 (\( p < 0.05 \)). As shown in Figs. S1–S3, gut microbiota were significantly negatively associated with DEGs of immune system process, inflammatory response, response to bacterium.

4. Discussion

To better understand the pathophysiology of TBI, we developed controlled cortical impact (CCI) models with a severe depth (Dixon et al., 1991); which provide reproducible and well-controlled injury to the brain. The model, minimizing the symptomology and outcomes following TBI, could be used to evaluate physiologic and functional deficits underlying acute and chronic TBI. This study was performed at three time points (1 d, 3 d and 7 d) after injury to induce acute and delayed responses. Although primary and secondary injury can roughly
be reproduced by TBI models, it fails to mimic all types of human brain injury. TBIs always remain multisystem disorders involving the central nervous system (CNS) and several peripheral tissues (Plesnila, 2016; Gyoneva and Ransohoff, 2015; Rasouli et al., 2011); especially the gastrointestinal system, thus, it is difficult to generate systematic models similar to human TBIs.

The current research focuses on the changes in the gut microbiota and brain transcriptome at 7 days following TBI. In previous studies, dysregulation of the intestinal flora and the mechanism of the brain-gut axis after TBI have been widely reported (Treangen et al., 2018). A study in 2021 by Wendong You et al also found that alpha diversity of the gut microbiome may change dynamically in a time-dependent manner after...
TBI. It gradually decreases after TBI, reaches the lowest level 24 h ~ 3 days after the injury, and then gradually recovers 3–7 days after the injury (You et al., 2021). Consistent with previous studies, our study also found intestinal flora disorder after TBI. After gut microbiota analysis at different levels (phylum, class, order, family, genus and species level), we found that the structure and composition of gut microbiota communities in the TBI group were significantly different from those in the sham group.

Although alpha and beta diversity of the TBI group and the sham group did not change significantly at 7 days after TBI, LEfSe analysis

Fig. 5. Predicted DEG functions based on GO and KEGG pathway analysis between the TBI and sham groups. A: Volcano plot; B: GO enrichment; C: KEGG pathway enrichment.
showed 35 differentially expressed strains in TBI mice. One of the most important strains is Bifidobacterium, which is a key member of the gut microbiome in human GABA production (Duranti et al., 2020), and recent studies have revealed that members of the gut microbiota are able to produce GABA, modulating the gut-brain axis response. Among members of the human gut microbiota, Bifidobacteria are well known to establish many metabolic and physiologic interactions with the host. A study in 2019 by Yuanyuan Ma et al. found that Lactobacillus may play a neuroprotective role by reshaping the intestinal microbiota of TBI mice (Ma et al., 2019). In 2018, H. Li also found that the neuroprotective effect of Clostridium butyricum on mice with TBI and its mechanism were partly attributed to the increased secretion of glucagon-like peptide-1 (GLP-1) in the intestinal brain axis (Li et al., 2018). Similarly, both Bifidobacterium and Lactobacillus can secrete GABA, so we can speculate that Bifidobacterium may also serve as a neuroprotective probiotic, but this needs to be confirmed by further studies. Overall, the gut

Fig. 6. Overview of significant DEGs in the Toll-like receptor signaling pathway of the inflammatory response between TBI and sham mice at 7 d postinjury. Cxcl9: chemokine (C-X-C motif) ligand 9; Ticam2: toll-like receptor adaptor molecule 2; Pik3cg: phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit gamma; Tlr1: toll-like receptor 1; Cxcl10: chemokine (C-X-C motif) ligand 10; Ccl5: chemokine (C–C motif) ligand 5; Tlr9: toll-like receptor 9; Ccl3: chemokine (C–C motif) ligand 3; Tlr7: toll-like receptor 7; Tlr6: toll-like receptor 6; Ccl14: CD14 antigen; Tlr4: toll-like receptor 4; Tlr2: toll-like receptor 2; Tlr13: toll-like receptor 13.

Fig. 7. Overview of significant DEGs in the chemokine signaling pathway of the inflammatory response between TBI and sham mice at 7 d postinjury. Ccr1: chemokine (C–C motif) receptor 1; Lyn: LYN proto-oncogene, Src family tyrosine kinase; Ccl12: chemokine (C–C motif) ligand 12; Cxcl9: chemokine (C-X-C motif) ligand 9; Ncf1: neutrophil cytosolic factor 1; Pik3cg: phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit gamma; Cxcl5: chemokine (C-X-C motif) ligand 5; Cx3cl1: chemokine (C-X3-C motif) ligand 1; Cxcl10: chemokine (C-X-C motif) ligand 10; Hck: hemopoietic cell kinase; Cxcl9: chemokine (C–C motif) ligand 9; Ccl6: chemokine (C–C motif) ligand 6; Ccl15: chemokine (C–C motif) ligand 5; Ccl3: chemokine C–C motif ligand 3; Ccr5: chemokine (C motif) receptor 5.
microbiome and daily feeding modalities may influence progress of TBI, and administration of probiotics may confer a health benefit to the damaged brain via gut-brain cross-talk, serving as a novel therapeutic target.

Using second-generation sequencing technology, we found 523 DEGs in the cerebral cortex in the TBI group compared with shams. Further analysis revealed that 93 genes related to immune system processes and 55 genes related to inflammation were differentially expressed. Our results showed that the immune process and inflammatory response of cells significantly changed after TBI, further aggravating the secondary injury of TBI, which is consistent with the neuropathological characteristics of TBI. The breakdown of the BBB after TBI allows peripheral immune and inflammatory cells to infiltrate the central nervous system. These white blood cells can be classified into cytokines, cytotoxic pro-teases and reactive oxygen species to activate the immune activity of intrinsic glial cells in the CNS (Nasser et al., 2016). Immune activation of microglia and astrocytes in the central nervous system leads to the production of cytokines and chemokines, and microglial activation is a key factor that can trigger cerebral hypoxic-ischemic inflammation (Yorger et al., 2019). The Toll-like receptor (TLR) pathway is an important component of the innate immune system after enrichment analysis of differential gene expression. Activation of the TLR pathway can lead to activation of different transcription factors, such as NF-κB and AP1, followed by the production of proinflammatory factors, such as interleukin and tumor necrosis factor (Shi et al., 2019). Some studies have found that TLR4-specific antagonists can inhibit neuro-inflammation by reducing the excessive production of inflammatory mediators. However, they may interfere with the protein clearance mechanism and myelination formation (Leitner et al., 2019). Hongsheng Jiang’s study in 2018 also found that TLR4 knockdown improves the neuroinflammatory response and further injury following brain injury by inhibiting the induction of autophagy and astrocyte activation. Therefore, TLR4 may be an important therapeutic target for neuroinflammatory injury after TBI (Jiang et al., 2018).

Evidences have demonstrated a link between brain trauma and intestinal dysfunction, the focus of attention has shifted to the performance of gut microbiota in immune-inflammatory response after brain trauma currently. After TBI, the levels of cytokines and chemokines released by microglia, astrocytes, cerebrovascular endothelial cells and peripheral immune cells are significantly increased (Forstner et al., 2018). In general, the immune-inflammatory response is critical for debris removal, repair, and regeneration after TBI, and this imbalance again causes acute and chronic brain damage (Yang et al., 2018). Cortical injury can lead to inflammatory consequences in the lining of the gastrointestinal mucosa, and gut microbiota can also influence brain function and behavior through the peripheral and central immune system. In the present study, we found altered structure and composition of gut microbiota communities in the TBI group. Differentially expressed strains, especially Bifidobacterium, were found significantly negatively associated with inflammatory response related cortical gene expression. Based on our results, we speculate a host-gut microbiota interaction and TBI-induced pathology following brain trauma, which represents a potential therapeutic target for TBI.

There are some limitations in this paper. Firstly, in the previous study, sex and age differences were reported to have impact on the gut microbiota composition (Org et al., 2016) and cortical gene expression (Trabzuni et al., 2013). The study used 8-week eight-week-old C57BL/6 mice to induce brain trauma, sex differences should be further considered. Secondly, we investigated the change of motor function after TBI, other sensorimotor tests in the next research are need to be performed for a better interpretation of brain injury. Thirdly, the authors explored the structure and composition of gut microbiota communities in CCI model, however, due to the complexity of the pathophysiology of TBI, CCI models have difficulty fully simulating the injury process, and further progress should be made with regard to new relevant models that better represent TBI patients. Fourthly, changes in cerebral cortex gene expression and gut microbiota composition after TBI have been revealed in the study, nevertheless, little was known about the relationship between changes in intestinal flora and cortical gene expression. The validation on protein level, the mechanism and pathway of action of the brain-gut axis are required in this field in the future. Additionally, the duration of the injury can be extended to study the changes in intestinal microflora and cerebral cortex gene expression after acute, subacute and chronic TBI.

Future research directions can be based on our transcriptome study to select genes with important functions and conduct experiments on mice lacking the related genes to observe whether the immune-inflammatory response of mice with gene knockout is reduced after TBI to further verify the accuracy of the results of this study.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.brainres.2022.148149.

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