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Aberrant anterior insular functional connectivity patterns and intestinal microbiota differences in mild traumatic brain injury patients accompanied by cognitive impairment

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Abstract

This study aimed to explore the correlation between differences in the abundance of gut microbiota and functional connectivity (FC) in the bilateral anterior insula (aIns) among patients with mild traumatic brain injury (mTBI). Twenty-six mTBI patients and forty-six age- and sex-matched healthy controls (HCs) were recruited for this study. Participants underwent clinical and neuropsychological assessments, resting-state functional MRI scans, fecal sample collection, and sequencing of intestinal microbial genes. Comparing the relative abundance of gut microbiota between mTBI patients and HCs revealed several bacterial genera showing increased or decreased abundance in mTBI patients.

Furthermore, disrupted FC was observed between the aIns and various regions (such as the left cerebellum Crus2, the orbital part of the right middle frontal gyrus (ORB.Mid.R), and the bilateral putamen) in mTBI. Particularly, decreased FC from the left aIns to the ORB.Mid.R, along with reduced levels of *Prevotella*, may collectively be associated with the onset or exacerbation of depression in mTBI patients. Notably, a potential shift characterized by an increase in *Bifidobacterium* and *Lactobacillus*, could be associated with brain damage and cognitive impairment among mTBI. These findings offer insights that could guide future clinical investigations towards the development of targeted probiotic formulations aimed at alleviating various mood disorders.

1. Introduction

Traumatic brain injury (TBI) represents a prevalent medical emergency worldwide, affecting nearly half of the global population at least once in their lifetime, with mild TBI (mTBI) constituting approximately 80% of cases(Maas, Menon, Adelson et al. 2017). Further, mTBI is associated with an elevated risk of developing neurodegenerative conditions(Perry, Sturm, Peterson et al. 2016), and recent focus has shifted towards assessing the relationship between cognitive function post-injury and brain functional connectivity (FC).

The intricate neural network within the cerebral cortex governs various human behaviors, habits, thoughts, and emotions(Liu, Wang, Yu et al. 2008), and functional magnetic resonance imaging (fMRI) during resting state offers a comprehensive view of this core network. According to the triple network model proposed by Menon(Menon and Uddin 2010), highlights the salience network (SN) as a crucial mediator among various networks(Xu, Seminowicz, Krimmel et al. 2022). The rising incidence of mTBI and post-traumatic stress disorder (PTSD) has sparked interest in studying the relationship between the anterior insula (aIns) and traumatic brain injuries. Previous investigations indicate that females experiencing PTSD following domestic violence history exhibit heightened hemodynamic activity in the right anterior insula (aIns.R)(Simmons, Paulus, Thorp et al. 2008). Similarly, severe PTSD correlates with increased FC in the aIns.R, with the extent of FC alterations directly proportional to PTSD severity(Simmons, Flagan, Wittmann et al. 2013). Notably, our prior research underscores a link between reduced FC in the anterior insula and diminished cognitive function in mTBI(Wang, Zhang, Sun et al. 2021). Nevertheless, the potential interplay of additional factors alongside aberrant aIns FC in

contributing to cognitive impairment among individuals with mTBI remains a topic requiring further exploration.

The gut microbiota has emerged as a critical modulator of behavior and cognitive functioning, influencing various processes such as pain perception(Amaral, Sachs, Costa et al. 2008) and stress responsiveness(Dinan and Cryan 2012). In the absence of trauma, some microbial differences could potentially impact vagal transmission to the brain, notably the insula region. For example, an fMRI study revealed that a 4-week supplementation of *Lactobacillus* and *Bifidobacterium* species induced modifications in insular brain activity during an emotional attention task among healthy females(Tillisch, Labus, Kilpatrick et al. 2013). Additionally, previous research has identified alterations in bacterial taxa attributed to smoking, particularly affecting *Bacteroides* and *Prevotella*, which may be associated with shifts in amino acid and neurotransmitter concentrations in the gut(Curtis, Stewart, Robinson et al. 2019). However, the precise alterations in gut microbiota abundance and their impact on neurocognitive functions in mTBI remain inadequately understood. Previous investigations utilizing a rodent model of TBI demonstrated rapid differences in gut microbiota composition within just 2 hours post-injury(Treangen, Wagner, Burns et al. 2018; Nicholson, Watts, Burmeister et al. 2019). Despite some advances, there exists a notable gap in research focusing on the interaction between gut microbiota, neurocognition, and mTBI patients.

To overcome these gaps, this study aimed to explore the potential involvement of post-mTBI gut microbiota differences in modulating the FC of the anterior insula. Our central hypothesis posited that alterations in both gut microbiota composition and aIns FC may occur in patients with mTBI during the acute phase. Furthermore, we aimed to establish a correlation between these differences and subsequent neurocognitive impairment in this population.

2. Materials and methods

2.1 Participants

Patients with mTBI treated at the 2nd Affiliated Hospital of Wenzhou Medical University from August 2016 to October 2018 were included in this study. All patients had a Glasgow Coma Scale (GCS(Teasdale and Jennett 1974)) score of 15 and negative findings on CT scans. They underwent MRI scans and clinical examinations within one week of the injury. Fecal samples were collected on the morning of the imaging exam. The inclusion criteria, based on the World Health Organization's Collaborating Centre for Neurotrauma Task Force, required patients to have: (i) an initial GCS score of 13–15; (ii) less than 30 minutes of loss of consciousness (LOC) and less than 24 hours of post-traumatic amnesia (PTA), with transient neurological abnormalities such as focal neurologic signs, seizures, or non-surgical intracranial lesions; (iii) a week or less since mTBI; and (iv) be right-handed and aged 18–60 years old. Patients who: (i) had LOC > 30 min, PTA > 24 h, GCS score < 13, or were younger than 18 or older than 60; (ii) had a history of TBI, neurological disorders, chronic psychiatric conditions, or substance abuse; (iii) showed structural abnormalities on neuroimaging; (iv) required intubation or

had skull fractures, sedation, abnormal organ function, active gastrointestinal diseases, or used alcohol or antibiotics/probiotics recently; or (v) had mTBI from penetrating craniocerebral injuries were excluded. At last, 46 sex- and age-matched healthy controls (24 males, 22 females) were included in the study.

All patients and control participants were recruited at a single center from the local emergency department. The control group presented to the emergency department for non–head trauma–related conditions. None had a history of traumatic brain injury, neurological or psychiatric disorders, or prior intracranial surgery. Informed consent was obtained from all participants, and the study was approved by the local ethics ethics committee in compliance with the Declaration of Helsinki.

2.2 Neuropsychological tests

All the participants underwent comprehensive cognitive assessments within 48 hours of post-mTBI as previously described(Kinnunen, Greenwood, Powell et al. 2011; Xu, Zhang, and Bai 2022). The neurocognitive assessments included: (i) measurement of the speed of processing cognitive information using the Trail Making Test Part A (TMA) and Digit Symbol Coding (DSC)(Jaeger 2018); (ii) assessment of the working memory and executive function using the Forward Digit Span (FDS) and Backward Digit Span (BDS)(Joy, Kaplan, and Fein 2004); (iii) Language Fluency (LF) Test for verbal fluency assessment; (iv) depression severity assessment using the Beck Depression Inventory-II (BDI) test; (v) PTSD check using the PTSD Checklist-Civilian (PCL-C); (vi) Post-concussive symptoms were measured using the Rivermead Post-Concussion Symptom Questionnaire (RPCS)(Niu, Bai, Sun et al. 2019). And (vii) Fatigue Severity Scale (FSS)(Krupp, LaRocca, Muir-Nash et al. 1989) as well as (viii) Insomnia Severity Index (ISI) assessment(Sadeghniiat-Haghighi, Montazeri, Khajeh-Mehrizi et al. 2014).

2.3 Collection of fecal samples and extraction of microbial DNA

Fecal samples were collected from all study participants within 7 days of the injury and immediately stored at -80°C. Microbial DNA was extracted using the Qiagen QIAamp DNA Stool Mini Kit (Qiagen, Germany) following the manufacturer’s instructions, with DNase-free RNase used to remove RNA contaminants. The extracted DNA was quantified using the PicoGreen Assay Kit (Thermo Fisher, Shanghai) and a Qubit 3.0 Fluorometer.

The V3-V4 region of the 16S rRNA gene was amplified from total fecal DNA using universal primers. The amplification process included an initial denaturation at 95°C for 5 min, followed by 25 cycles of incubation at 95°C for 30 s, 50°C for 30 s, and 72°C for 40 s, concluding with a final extension at 72°C for 7 min. Library preparation and sequencing were carried out at Beijing Biomarker Technologies Co., Ltd using the Illumina HiSeq 2500 Platform (Beijing, China).

2.4 MRI data

Non-contrast CT scans were acquired using a 64-row CT scanner (LightSpeed VCT, GE Healthcare, Milwaukee, WI, USA) whereas MRI scans were acquired using a 3.0T MRI scanner (3.0T GE750, GE

Healthcare). All participants were instructed to remain calm and relaxed not to engage in any mental activities, and to keep their eyes closed. MRI was performed using the high-resolution T1-weighted 3D MPRAGE sequence (time of echo (TE) of 3.17 ms, time repetition (TR) of 8.15 ms, flip angle of 9°, slice thickness of 1 mm, the field of view (FOV) of 256 mm × 256 mm, and matrix size of 256 × 256). Single-shot, gradient-echo planar imaging sequence with 54 slices covered the whole brain with the following parameters: TR = 2,000 ms, TE = 30 ms, slice thickness = 3 mm, flip angle = 90°, FOV = 216 mm × 216 mm, matrix size = 64 × 64, volume size = 3 mm × 3 mm × 3 mm. The parameters of axial flow attenuated inversion recovery (FLAIR) are as follows: TR = 9,000 ms, TE = 95 ms, flip angle = 150°, thickness = 5 mm, slices = 20, FOV = 240 mm × 240 mm, and matrix size of 173 × 256.

2.5 Preprocessing of the resting-state fMRI data

The resting-state functional MRI data were analyzed using the Data Processing Assistant for Resting-State fMRI (DPARSF version 2.3) package in the MATLAB software. The first 10 frames were discarded to avoid hemodynamic delay. The preprocessing of the image data included time series correction, head movement correction (head movement in x, y, or z direction translation < 2.5mm or rotation of the three axes < 2.5°), partial registration, and normalization of anatomical and functional images into the standard brain space. The analysis was performed at the Montreal Neurological Institute (MNI) (size of voxel after interpolation =3mm^3). Then spatial smoothing (6-mm full-width-at-half-maximum), linear drift removal, and bandpass filtering (0.01-0.1Hz) were also performed.

2.6 Seed-based FC analysis

The bilateral anterior insula was used as seeds with a radius of 6 mm for network analysis (MNI coordinates(Dambacher, Sack, Lobbestael et al. 2015): left (-35, 16, 12) and right (29, 24, 9)). The relationship between the bilateral anterior insula and other voxels of the whole brain was calculated using the average Blood Oxygen Level-Dependent signal time series. Fisher z-transformation of the correlation coefficients was then performed to normalize the data. The seed-based FC maps were then generated.

2.7 Statistical analyses

Data analysis was conducted using SPSS software (version 23, IBM, Armonk, NY, USA). Continuous data were presented as mean ± standard deviation (SD), frequencies, and percentages. The Shapiro-Wilk test was used to assess normal distribution of data. Differences between groups, for normally and non-normally distributed data, were analyzed with the independent two-sample t-test and Mann-Whitney U test, respectively. Categorical variables were assessed using the Chi-square test. Group differences in functional connectivity (FC) were evaluated using Voxel-wise two-sample t-tests with an AlphaSim correction ($P < 0.05$, combined threshold of $P < 0.01$ and minimum cluster size of 40 voxels). The relationship between gut microbiota abundance, clinical scales, and FC differences was examined using the Spearman rank correlation test while controlling for factors such as age, gender, education, body mass index (BMI), smoking, drinking, and defecation habits. Statistical significance was set at $P < 0.05$.

Given the relatively small cohort and the exploratory nature of these secondary analyses in this pilot investigation, uncorrected findings are presented to avoid overly conservative thresholds that might obscure potentially meaningful signals.

3. Results

3.1 Demographics and initial neuropsychological data

A total of 26 patients with mild traumatic brain injury (mTBI) (mean age 42.96 ± 13.95 years; 13 females) and 46 healthy controls (HCs) (mean age 41.62 ± 12.04 years; 22 females) were recruited for this study. There were no significant differences in age, education level, or gender between the two groups (all $P > 0.05$). The demographic and clinical profiles of all participants are detailed in Table 1. The initial Glasgow Coma Scale (GCS) score for all mTBI patients was 15. The injuries were predominantly caused by car accidents (53%), assaults (23%), and falls (23% ground-level falls and falls from heights of 2–3 meters). Patients exhibited lower scores in Backward Digit Span (BDS) ($P = 0.018$) and Digit Symbol Coding (DSC) ($P = 0.028$) compared to HCs, whereas scores in Reaction to Preconscious Stress (RPCS), PTSD Checklist-Civilian (PCL-C), Beck Depression Inventory (BDI), and Insomnia Severity Index (ISI) were notably higher in patients (all $P < 0.001$, see Table 1).

Table 1
Demographics and neuropsychological scales of all participants

Demographic	mTBI patients	HCs	*P* values
Age(Y)	42.96 ± 13.95	41.62 ± 12.04	0.462
Gender(M/F)	13/13	24/22	0.258
Education(Y)	8.24 ± 4.07	9.89 ± 6.18	0.062
BMI(kg/m2)	23.02 ± 3.70	22.91 ± 3.17	0.47
Neuropsychological test			
TMA(s)	75.52 ± 4.68	61.47 ± 42.17	0.462
FDS	7.60 ± 1.26	8.11 ± 1.86	0.232
BDS	3.68 ± 0.90	4.69 ± 1.89	0.018*
LF	15.44 ± 5.52	16.71 ± 5.59	0.432
DSC	31.44 ± 16.83	41.38 ± 18.13	0.028*
Self-reported assessment			
RPCS	12.52 ± 10.16	1.49 ± 2.13	<0.001*
PCL-C	26.48 ± 7.68	17.0 ± 0.0	<0.001*
FSS	9.0 ± 0.0	9.0 ± 0.0	1
BDI	6.04 ± 4.52	0.02 ± 0.14	<0.001*
ISI	7.28 ± 6.13	1.73 ± 2.63	<0.001*
MTBI severity N (%)			
GCS = 15	26(100%)		
GCS = 13,14	0(0%)		
Causes for mTBI N (%)			
Car accident	14(53%)		
assault	6(23%)		
falls	6(23%)		

3.2 Characteristics of gut microbiota in mTBI

The gut microbiota characteristics of patients with mTBI in the current study were based on an unpublished report in our team, comprising 98 mTBI patients and 62 matched healthy controls. Notably, at the phylum level, there was a significant reduction in the abundance of *Firmicutes* (P = 0.011, see Fig. 1). When examining the bacterial composition at the genus level using linear discriminant analysis (LDA) effect size analysis, 17 bacterial taxa showed significant differences in relative abundance between mTBI patients with LDA scores > 3.0 and healthy controls, after adjusting for confounding factors such as age, gender, BMI, smoking, drinking, and bowel habits (P < 0.05). Specifically, the abundance of 8 bacteria including Escherichia, Shigella, Ruminococcus, Bifidobacterium, Muribaculaceae, Lactobacillus, Clostridium, Catenibacterium, and Alloprevotella increased, while the levels of 9 bacteria including Prevotella_7, Dorea, Dialister, Eubacterium_ruminantium, Fusicatenibacter, Lachnospiraceae_NK4A136_group, Fusobacterium, Agathobacter, and Faecalibacterium all decreased.

3.3 Abnormal resting-state functional connectivity

A significant reduction in functional connectivity (FC) was observed in specific brain regions of patients with mTBI within bilateral anterior insula (aIns) regions (refer to Fig. 2). Connectivity from the left aIns to the left cerebellum Crus2, right middle frontal gyrus (ORB.Mid.R), and left putamen, as well as from the right aIns to the right putamen, exhibited lower levels in mTBI patients (refer to Table 2).

Table 2
Altered insula functional connectivity in mTBI

Nodes	**Mean Ps ±SD**	**Mean Cs ±SD**	***P* values**	**MNI**		
				x	**y**	**z**
aIns.L→ Cerebelum.Crus2.L	-3.43 ± 17.07	13.10 ± 17.78	0.001	-33	-75	-78
aIns.L→ ORB.Mid.R	-4.78 ± 7.60	4.34 ± 8.45	< .001	42	54	-15
aIns.L→ Putamen.L	29.40 ± 12.46	46.21 ± 21.04	0.001	-21	6	15
aIns.R→ Putamen.R	35.05 ± 20.42	49.68 ± 18.73	0.014	30	0	-3

3.4 Relationships between the abundance of gut microbiota, abnormal functional connectivity, and neuropsychological parameters

The correlation analysis examining the relationship between the gut microbiota composition and abnormal brain function in the bilateral anterior insula regions is detailed in Table 3. A significant positive correlation was identified between BDI scores and the FC from the left aIns to the ORB.Mid.R (r = 0.430, P = 0.036) in patients with mTBI. Furthermore, a significant positive correlation was found between the DSC score and the abundance of Bifidobacterium (r = 0.514, P = 0.010, see Fig. 3). Lastly, a positive correlation was observed between the abundance of *Prevotella_7* and the ORB.Mid.R in individuals with

mTBI (r = 0.532, P = 0.028). Detailed relationships are visually summarized in the heatmap presented in Fig. 4.

Table 3
Significant correlation between gut microbiota, neuropsychological scales, and abnormal brain traits in mTBI.

Scales/brain regions	Bacterial genus	r	*P* values
DSC	Bifidobacterium	0.514	0.010
ORB.Mid.R	Prevotella_7	0.532	0.028

4. Discussion

Lactobacillus and *Bifidobacterium*, are generally considered beneficial probiotics, what is unexpected is that, certain strains within these genera can become pathogenic under specific circumstances. Interestingly, we exactly observed an increase in *Bifidobacterium* and *Lactobacillus* in the gut of mTBI patients, both bacteria are Gram-positive and can ferment cellulose into pathogens through symbiosis. Similar "adverse effects" of probiotics have been previously reported in some literature. For example, *Lactobacillus casei* has been implicated in bacteremia and infective endocarditis(Sherid, Samo, Sulaiman et al. 2016), and increased levels of *Lactobacillus* have been linked to Graves' disease(Jiang, Yu, Kosik et al. 2021), which through the reverse activation of the NF-κB signaling pathway(Chang, Yarkoni, Khaw et al. 2013). Studies in rat models have shown elevated levels of *Lactobacillus* and *Bifidobacterium* following spinal cord injury(Littman and Pamer 2011; O'Connor, Jeffrey, Madorma et al. 2018), suggesting that probiotics may not always have positive effects. It is possible that the rise in *Bifidobacterium* and *Lactobacillus* post-injury represents a compensatory mechanism to restore gut microbiota balance. Further research with larger sample sizes is required to validate these explanations.

The anterior insula (aIns) is known for housing specialized nodes that regulate cognitive and motor responses(Seeley, Menon, Schatzberg et al. 2007), thereby the alterations in cognitive and motor functions have a great possibility link to disruptions in functional connectivity within the aIns. Our study revealed decreased FC from the left aIns to the left cerebellum Crus2, the ORB.Mid.R, and the left putamen in mTBI patients. Additionally, notably reduced FC was observed from the right aIns to the right putamen. Previous research by Bagga et al.(Bagga, Reichert, Koschutnig et al. 2018) highlighted a correlation between self-reported behavioral measures and differences in brain activation in the cerebellum when probiotics were consumed by healthy individuals, indicating the influence of probiotics on decision-making and memory processes. Therefore, it is possible that the "adverse effects" of *Bifidobacterium* and *Lactobacillus* after injury in mTBI patients is responsible for their cognitive decline, as indicated by a low DSC score. Meanwhile, patients with mTBI also exhibited higher scores in measures related to motivation and emotion (RPCS and PCL-C), potentially due to altered connectivity in the putamen affecting their response to these factors, which plays a role in regulating motivation, reward, and emotional processes.

In the present study, we found a positive correlation between ORB.Mid. R connectivity and BDI, which suggests the patients with reduced FC in this region may have depression-related problems. The frontal cortex, particularly the middle frontal gyrus, is associated with problem-solving, reasoning, attention, learning, and creativity(Zhang, Ide, and Li 2012; Chica, Bartolomeo, and Lupiáñez 2013; Xu, Chen, Tao et al. 2022). Notably, it has been documented that disruptions in the orbitofrontal cortex and the amygdala, key components of the limbic system(Bechara, Tranel, Damasio et al. 1996), may be associated with increased impulsivity and loss of control in mTBI patients. Surprisingly, some studies have even found a negative correlation between FC in the left amygdala and the ORB.Mid.R, associated with a decreased risk of suicide(Zhu, Tian, Wang et al. 2020), aligning with the findings of this study. Perhaps, the findings in this paper may serve to validate this perspective in the existing literature. Future studies may put the highlight on the importance of diagnosis and treatment of mTBI-induced depression.

In mTBI patients, our study found that pro-inflammatory *Escherichia_Shigella* increased, while anti-inflammatory *Prevotella_7* and *Faecalibacterium* decreased, disrupting the inflammation to neuro-protection balance. Numerous studies have highlighted a decrease in the relative abundance of the *Prevotella* genus in individuals with depression(Cheung, Goldenthal, Uhlemann et al. 2019; Valles-Colomer, Falony, Darzi et al. 2019; Bastiaanssen, Cussotto, Claesson et al. 2020). Moreover, studies have demonstrated a significant reduction in *Prevotella* levels in children with autism(Kang, Park, Ilhan et al. 2013), with these differences being more closely linked to the presence of autistic symptoms rather than the severity of gastrointestinal issues. In our study, we identified a positive correlation between FC from the left anterior insula to the right middle orbitofrontal cortex, *Prevotella* levels, and depression scores. This suggests that the decline in *Prevotella* abundance and the functional connectivity of the anterior insula may collaboratively contribute to the development of depressive symptoms. These results underscore the importance of advancing clinical research to explore whether probiotic interventions could serve as a viable alternative or adjunct strategy for managing depression and mood disorders.

Several limitations exist in this study that should be addressed for future research endeavors. Firstly, the cross-sectional design means that our findings only demonstrate strong correlations between gut microbiota differences and alterations in brain connectivity and cognitive outcomes. Consequently, longitudinal studies are essential to elucidate the long-term alterations in gut microbiota following mTBI. Secondly, given the relatively small sample size and the exploratory nature of the secondary analyses in this pilot investigation, uncorrected findings were presented to avoid overly conservative thresholds that might obscure potentially meaningful signals. While this approach prioritizes hypothesis generation, it increases the risk of false positives. We should call for larger scale research to corroborate our findings. Additionally, precise insula localization can better detect cognitive impairment. Furthermore, as the study relied on self - reported measures, data interpretation should be more cautious. Lastly, the characterization of gut microbiota in mTBI patients was obtained from secondary sources, potentially introducing inaccuracies. Future research should conduct longitudinal studies, use larger sample sizes, and precisely localize the insula to better understand the long-term effects of mTBI on gut microbiota and brain function.

5. Conclusions

This study revealed that patients with mTBI experienced a significant reduction in FC between the left anterior insula and the right middle orbitofrontal cortex. Additionally, a decrease in the abundance of *Prevotella* was associated with the development or exacerbation of depression in mTBI. Although these observations only establish associations between gut microbiota abnormalities, brain connectivity disruptions, and clinical outcomes in patients with mild traumatic brain injury (mTBI), the present findings can provide valuable guidance for designing more targeted clinical investigations ahead. Researchers may now focus on determining whether specific patterns of altered activity in key brain regions, alongside distinctive microbial signatures, can function as early predictive biomarkers. Identifying these markers could enable clinicians to forecast which individuals with mTBI are at heightened risk of developing depression or other persistent mood disorders in the post-injury period, ultimately opening avenues for timely preventive interventions.

Abbreviations

aIns
anterior insula
aIns.L
left anterior insula
aIns.R
right anterior insula
BDI
Beck Depression Inventory-II
BDS
Backward Digit Span
BMI
body mass index
DPARSF
Data Processing Assistant for Resting-State fMRI
DSC
Digit Symbol Coding
FC
functional connectivity
FDS
Forward Digit Span
FLAIR
flow attenuated inversion recovery
fMRI
functional magnetic resonance imaging
FOV

field of view
FSS
Fatigue Severity Scale
GCS
Glasgow Coma Scale
HCs
healthy controls
ISI
Insomnia Severity Index
L
left
LDA
linear discriminant analysis
LF
Language Fluency
LOC
loss of consciousness
MNI
Montreal Neurological Institute
MRI
Magnetic Resonance Imaging
mTBI
mild traumatic brain injury
ORB.Mid.R
orbital part of the right middle frontal gyrus
PCL-C
PTSD Checklist-Civilian
PTA
post-traumatic amnesia
PTSD
post-traumatic stress disorder
R
right
RPCS
Rivermead Post-Concussion Symptom Questionnaire
SD
standard deviation
SN
salience network
TBI

Traumatic brain injury
TE
time of echo
TMA
Trail Making Test Part A
TR
time repetition

Declarations

Ethics approval and consent to participate:

This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University. Written informed consent was obtained from all participants.

Consent for publication:

Not applicable.

Competing interests:

All authors have no conflicts of interest to declare.

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Author Contribution

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.Guanghui Bai was involved in the conception and design of the research topic, supervised the study, and revised the manuscript. LI YL and ZHAO PH contributed to data handling, aspects of data analysis, and manuscript preparation. CHEN J and Linkai Chen completed the writing and framing of the manuscript. Tianhui Li and YIN B collected and organized the data. All authors contributed to the article and approved the submitted version.

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Not applicable.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

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Figures

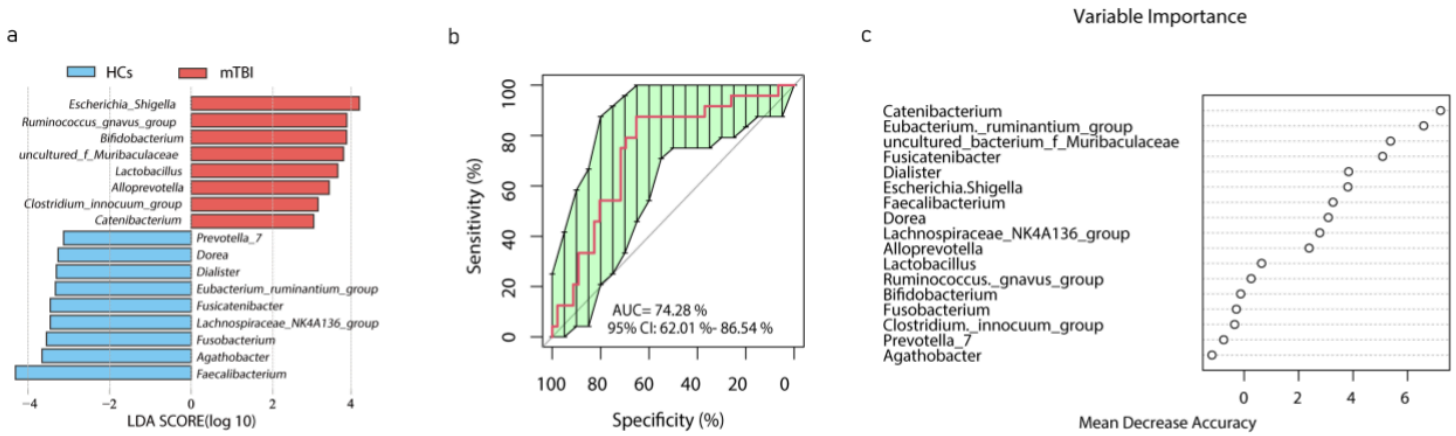


Figure 1

Differences of the abundance of gut microbiota in patients with mTBI. There was a significant difference in the relative abundance of bacteria between the mTBI patients and HCs after adjustment for age, sex, body mass index, smoking, drinking, and defecation habits. The main finding was the increase in *Bifidobacterium* and *Lactobacillus*, but the decrease in *Prevotella*. mTBI: mild traumatic brain injury; HCs: healthy controls; LDA: linear discriminant analysis.

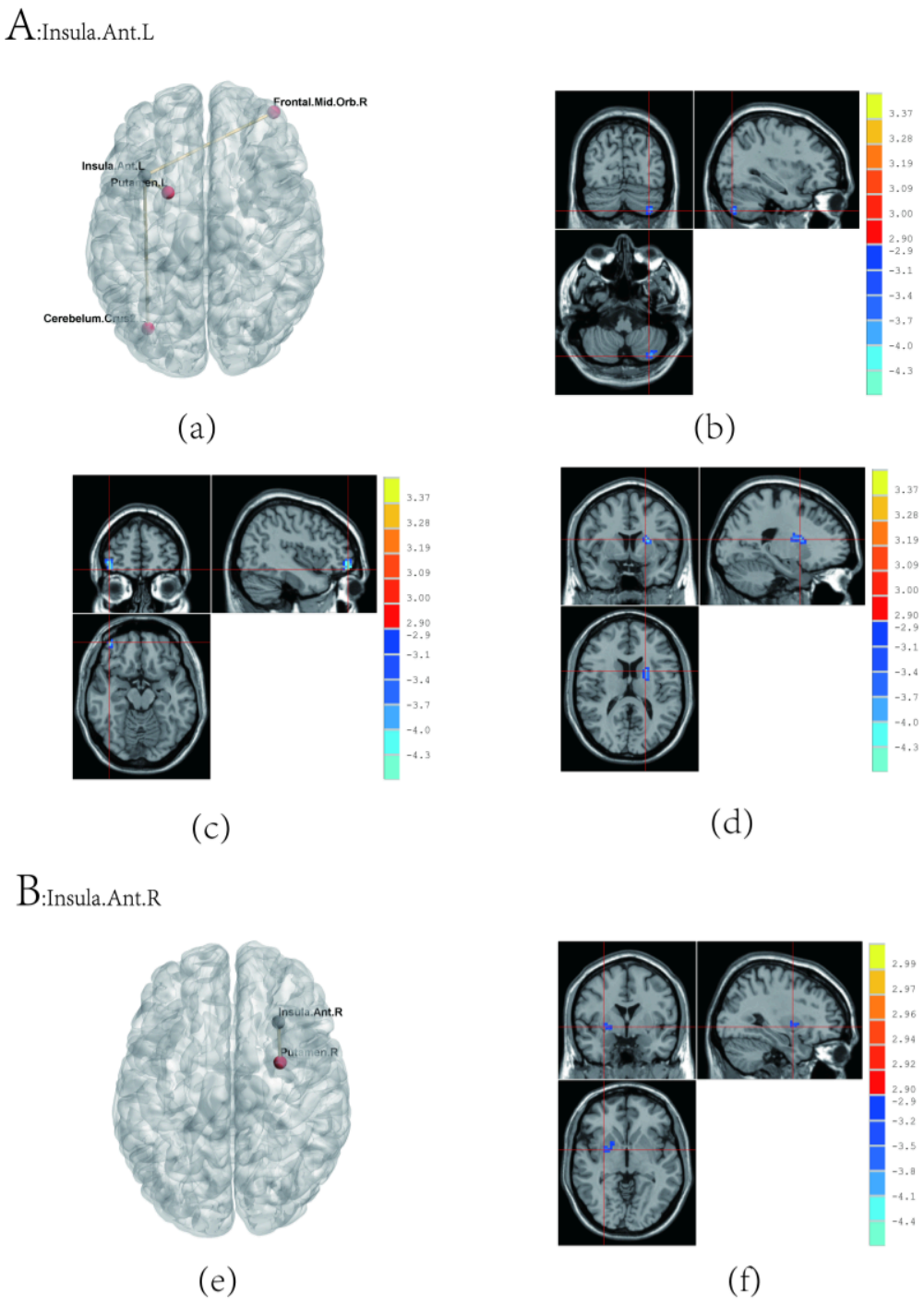


Figure 2

The difference between mTBI patients and HCs in resting-state FC. Taking bilateral anterior insula as the seed point, the FC decline was found from Insula.Ant.L to three brain regions, and from Insula.Ant.R to putamen, respectively (all at $P < 0.05$). (A) from the Insula.Ant.L to (b) Cerebelum.Crus2.L; to (c) ORB.Mid.R; to (d) putamen.L; (B) from Insula.Ant.R to (f) putamen.R. mTBI: mild traumatic brain injury;

HCs: healthy controls; FC: functional connectivity; Insula.Ant: anterior insula; ORB.Mid.R:middle frontal orbital gyrus; L: left; R: Right.

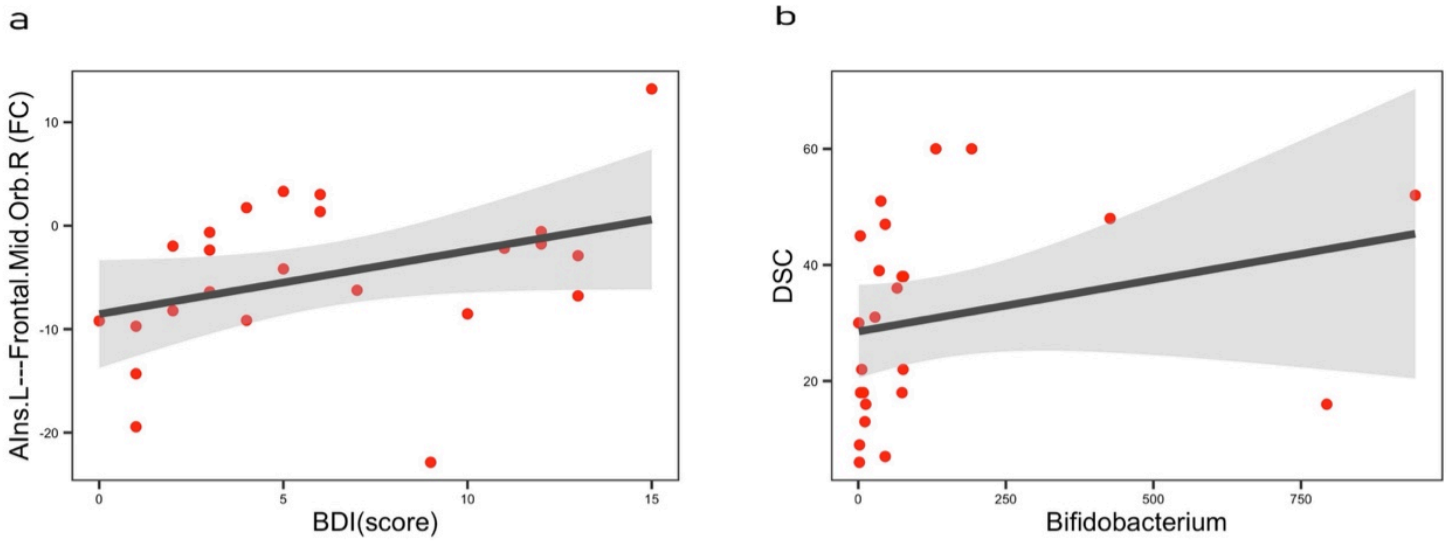


Figure 3

The correlation between brain function parameters, gut microbiota and specific neuropsychological performance in mTBI patients. (a) BDI score is positively correlated with the FC from aIns.L to Frontal.Mid.Orb; (b) *Bifidobacterium*was positively correlated with DSC. mTBI: mild traumatic brain injury; BDI: Beck Depression Inventory; FC: functional connectivity; aIns: anterior insula; Frontal.Mid.Orb: middle frontal orbital gyrus; DSC: Digit Symbol Coding; L: left; R: Right.

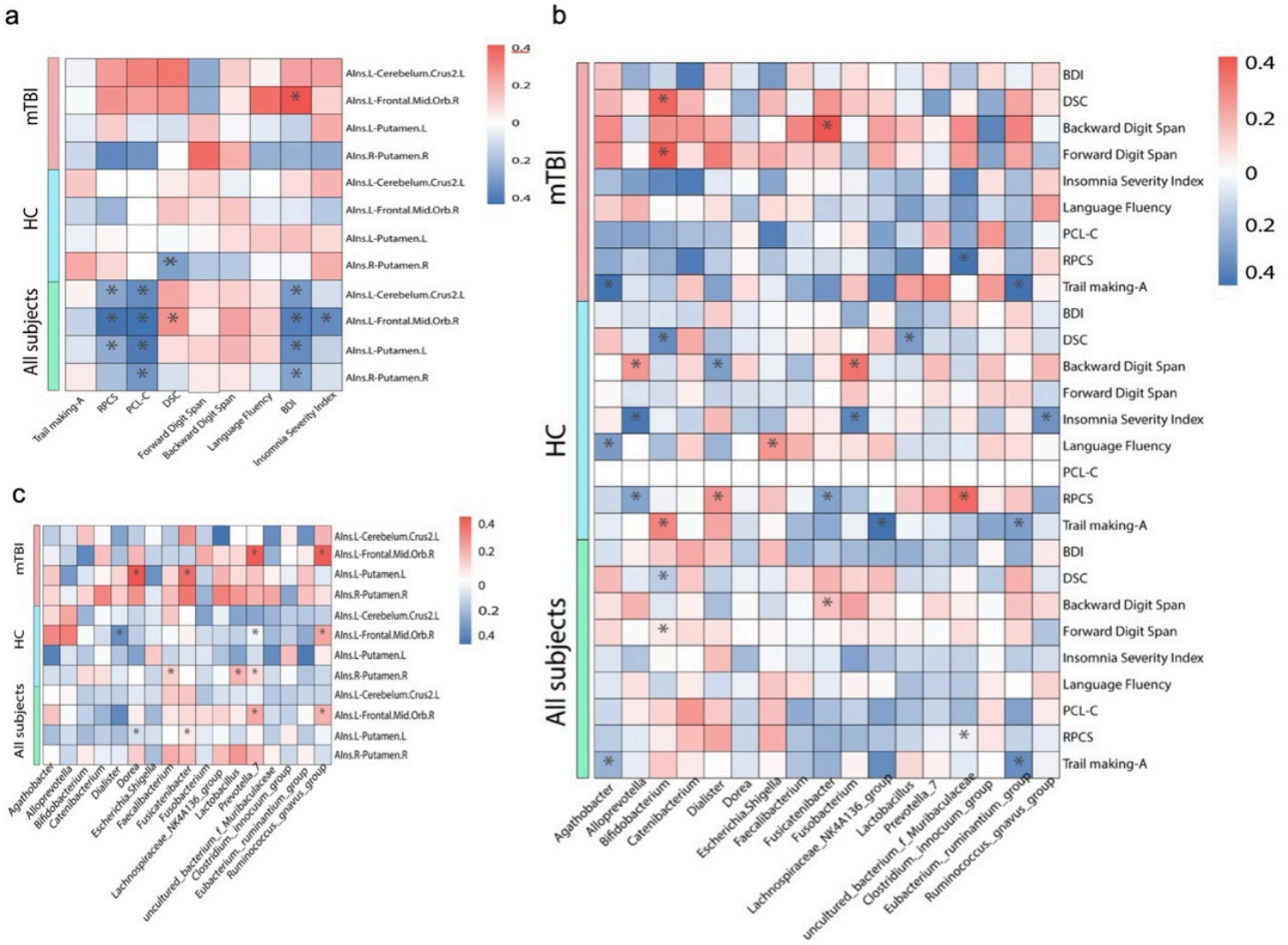


Figure 4

The associations between the abundance of gut microbiota, and neuropsychological performance and FC of aIns in mTBI patients. * indicates a correlation between the gut microbiota, neuropsychological performance and brain parameters. FC: functional connectivity; aIns: anterior insula; mTBI: mild traumatic brain injury.