Brain, Behavior, and Immunity xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Altered fecal microbiota composition in patients with major depressive disorder

Haiyin Jiang [a,1], Zongxin Ling [a,1], Yonghua Zhang [b,1], Hongjin Mao [c], Zhanping Ma [d], Yan Yin [c], Weihong Wang [e], Wenxin Tang [c], Zhonglin Tan [c], Jianfei Shi [c], Lanjuan Li [a,2], Bing Ruan [a,*]

[a] Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, the First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, Zhejiang 310003, China
[b] Department of Traditional Chinese Medicine, The Seventh People's Hospital of Hangzhou, Hangzhou, Zhejiang 310003, China
[c] Department of Psychiatry, The Seventh People's Hospital of Hangzhou, Hangzhou, Zhejiang 310003, China
[d] Department of Psychiatry, Psychiatric Hospital of Hengshui, Hebei 053000, China
[e] Department of Infectious Diseases, Huzhou Central Hospital, Huzhou, Zhejiang 313000, China

ARTICLE INFO

Article history:
Received 29 July 2014
Received in revised form 29 March 2015
Accepted 29 March 2015
Available online xxxx

Keywords:
Depression
Gut bacteria
Inflammation
Gut–brain
Antidepressant

ABSTRACT

Studies using animal models have shown that depression affects the stability of the microbiota, but the actual structure and composition in patients with major depressive disorder (MDD) are not well understood. Here, we analyzed fecal samples from 46 patients with depression (29 active-MDD and 17 responded-MDD) and 30 healthy controls (HCs). High-throughput pyrosequencing showed that, according to the Shannon index, increased fecal bacterial α-diversity was found in the active-MDD (A-MDD) vs. the HC group but not in the responded-MDD (R-MDD) vs. the HC group. Bacteroidetes, Proteobacteria, and Actinobacteria strongly increased in level, whereas that of Firmicutes was significantly reduced in the A-MDD and R-MDD groups compared with the HC group. Despite profound interindividual variability, levels of several predominant genera were significantly different between the MDD and HC groups. Most notably, the MDD groups had increased levels of Enterobacteriaceae and *Alistipes* but reduced levels of *Faecalibacterium*. A negative correlation was observed between *Faecalibacterium* and the severity of depressive symptoms. These findings enable a better understanding of changes in the fecal microbiota composition in such patients, showing either a predominance of some potentially harmful bacterial groups or a reduction in beneficial bacterial genera. Further studies are warranted to elucidate the temporal and causal relationships between gut microbiota and depression and to evaluate the suitability of the microbiome as a biomarker.

1. Introduction

Depression is a common, life-disrupting and highly recurrent illness and a leading source of disability worldwide (Moussavi et al., 2007). Despite the fact that antidepressant medication is widely used to treat depressive symptoms, 30–40% of patients do not respond to current drug strategies (Rush et al., 2006). Studies on depression have focused mainly on the genetic, behavioral, and neurological aspects of the disease, although the contributions of environmental risk factors and immune dysregulation to the etiology of depression have gained significant attention.

Accumulating evidence from animal studies supports the hypothesis that gut microbiota play an important role in central nervous system function, namely through inflammation, and the hypothalamic–pituitary–adrenal (HPA) axis, and by affecting neurotransmission (Bangsgaard Bendtsen et al., 2012; Collins et al., 2012; Cryan and Dinan, 2012; Dinan and Cryan, 2013; Dinan et al., 2013; Wang and Kasper, 2014). Although the pathways linking gut bacteria with the brain are incompletely understood, "leaky gut", induced by stress, could play a role (Rook and

Abbreviations: BDNF, brain-derived neurotrophic factor; DSM-IV, the Diagnostic and Statistical Manual of Mental Disorders-Fourth Edition; HCs, healthy controls; HPA, hypothalamic–pituitary–adrenal; IL-6, interleukin-6; IL-1β, interleukin-1 beta; HAMDS, Hamilton's Depression Scale; MADRS, Montgomery–Asberg Depression Rating Scale; MDD, major depression disorder; TNF-α, tumor necrosis alpha.

* Corresponding author at: Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, the First Affiliated Hospital, School of Medicine, Zhejiang University, Qingchun Road No. 79, Hangzhou 310003, China. Tel.: +86 571 8723 6458; fax: +86 571 8723 6585.
E-mail addresses: ljli@zju.edu.cn (L. Li), hzruanbing@gmail.com (B. Ruan).
[1] These authors contributed equally to this work.
[2] Co-corresponding author. Tel.: +86 571 8723 6585; fax: +86 571 8723 6459.

http://dx.doi.org/10.1016/j.bbi.2015.03.016
0889-1591/

Lowry, 2008; Rook et al., 2014). Specifically, increased translocation of bacterial products, due to a compromised gut barrier has been linked to activation of the immune system and HPA axis (Maes et al., 2012, 2013). These effects can be reversed in mice by oral administration of probiotics (Ait-Belgnaoui et al., 2012, 2014; Savignac et al., 2014). In line with these findings, human studies have demonstrated increased bacterial translocation in mood disorders such as depression (Maes et al., 2008, 2012). Indeed, recent reports of trials using probiotics in healthy subjects demonstrated improvements in depression or anxiety outcome measures (Messaoudi et al., 2011).

These results indicate that gut bacteria could possess therapeutic potential for mental illnesses. Within the past year, accumulating experimental data has strongly supported the view that depression could also influence microbiota composition. O'Mahony et al. (2009) reported that the fecal microbiome composition of adult rats subjected to maternal separation was significantly altered compared with that of non-separated controls. Bangsgaard Bendtsen et al. (2012) demonstrated that the community microbiota structure of mice exposed to a prolonged restraint stressor differed significantly from that of non-stressed controls. Bailey et al. (2011) observed that social stressors significantly altered the relative abundance of bacteria, particularly when microbiota were assessed immediately following stressor exposure. Despite such animal studies indicating an association between gut microbiota and depression, this relationship remains poorly understood in humans. Therefore, detailed assessment of the fecal microbiota of depression patients should be undertaken before firm conclusions are drawn.

Here, we investigated whether gut microbiota are altered during major depressive episodes or in response to antidepressant treatment. We also identified microbiota signatures specific for depression and their relationships with clinical patterns and physiological measures using massively parallel barcoded 454 pyrosequencing on 76 fecal samples taken from 46 patients diagnosed with depression and 30 matched healthy controls (HCs).

2. Methods

2.1. Subject selection

This study protocol was approved by the Ethics Committee of The Seventh People's Hospital of Hangzhou (Zhejiang, China). After receiving a written description of the aim of this study, all participants gave written informed consent prior to enrollment. The recruitment of participants and the process of sample collection are depicted in Fig. 1.

Forty-six patients (age, 18–40 years) were ultimately recruited from the Seventh People's Hospital of Hangzhou in Hangzhou, Zhejiang, from May 2013 to December 2013 (Table 2). One experienced psychiatrist performed the screening examinations. The Mini-International Neuropsychiatric Interview was used as a systematic psychiatric screening tool to detect preexisting psychiatric disorders (Sheehan et al., 1998). The Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders-Fourth Edition was used to verify major depressive disorder (First MB). Participants completed the Hamilton's Depression Scale (HAMDS), a 24-item clinician-administered measure of depression, to assess the severity of depressive symptoms (Hamilton, 1960). The HAMDS generates scores from 0 to 63; scores $\geqslant$20 are indicative of clinically significant depression. Depressive symptoms were also assessed using the Montgomery–Asberg Depression Rating Scale (MADRS), a clinician-related depression scale and one of the most commonly used symptom severity scales for depression. It consists of 10 items scored from 0 to 6 (Montgomery and Asberg, 1979). HC subjects (n = 30) from the same cohort were screened using a semi-structured clinical interview to exclude those with psychiatric or physical illnesses.

All subjects were examined clinically before sampling and were subsequently divided into three groups: active-MDD (A-MDD) group (n = 29), responding-MDD (R-MDD) group (n = 17), and HCs (n = 30). The A-MDD group was defined as having an HAMDS score $\geqslant$20. The patients in the R-MDD group were defined as those with a baseline HAMDS scores $\geqslant$20 upon admission to the hospital. Fecal and serum samples were collected at the time of their HAMDS scores showed a 50% reduction after 4 weeks treatment.

The following exclusion criteria were established: hypertension; cardiovascular disease; diabetes mellitus; obesity; liver cirrhosis; fatty liver disease; irritable bowel syndrome; inflammatory bowel disease; drug or alcohol abuse in the last year; use of antibiotics, probiotics, prebiotics, or synbiotics in the month before collection of the fecal sample; and known active bacterial, fungal, or viral infections.

2.2. Fecal sample collection and DNA extraction

Fecal samples were collected in a sterile plastic cup after the participants completed the HAMDS and MADRS assessments and were kept in an icebox. Samples for bacterial genomic DNA extraction were delivered to the laboratory within 15 min and stored at −80 °C. Fecal microbial DNA was extracted from 200-mg feces using the QIAamp® DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions, with the additional glass-bead beating steps on a Mini-beadbeater (FastPrep; Thermo Electron Corp., Boston, MA, USA). DNA was quantified using a NanoDrop ND-1000 spectrophotometer (Thermo Electron); integrity and size were assessed by 1.0% agarose gel electrophoresis on gels containing 0.5 mg/mL ethidium bromide. DNA was stored at −20 °C before analysis.

2.3. Serum cytokine and BDNF detection

Blood samples were collected immediately after the HAMDS and MADRS assessment, transferred to the laboratory immediately in an icebox and stored at −80 °C within 15 min after preparation for further analysis. Serum tumor necrosis factor-α (TNF-α), interleukin-1β (IL)-1β, IL-6, and brain-derived neurotrophic factor (BDNF) levels were determined using commercially available enzyme-linked immunosorbent assay kits (RayBiotech, Norcross, GA, USA).

2.4. Polymerase chain reaction (PCR) and pyrosequencing

Triplicate PCR reactions were performed on each sample. The bacterial genomic DNA was amplified with the 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 533R (5′-TTACCGCGGCTGCTGGCAC-3′) primers specific for the V1–V3 hypervariable regions of the 16S rRNA gene. Each forward primer incorporated FLX Titanium adapters and a sample barcode at the 5′ end of the reverse primer to allow all samples to be included in a single 454 FLX sequencing run (Table S1). All PCRs were performed in 50-μL triplicates and combined after PCR. The products were extracted with the QIAquick Gel Extraction kit (Qiagen) and quantified on a NanoDrop ND-1000 spectrophotometer, QuantiFluor-ST fluorometer (Promega, Madison, WI, USA), and an Agilent 2100 bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). Equimolar concentrations of the 76 samples were pooled and sequenced on a 454 Life Sciences genome sequencer FLX system (Roche, Basel, Switzerland) according to the manufacturer's recommendations.

Please cite this article in press as: Jiang, H., et al. Altered fecal microbiota composition in patients with major depressive disorder. Brain Behav. Immun. (2015), http://dx.doi.org/10.1016/j.bbi.2015.03.016