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Role of *Actinobacteria* and *Coriobacteriia* in the antidepressant effects of ketamine in an inflammation model of depression

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ABSTRACT

Ketamine, an *N*-methyl-D-aspartic acid receptor (NMDAR) antagonist, elicits rapid-acting and sustained antidepressant effects in treatment-resistant depressed patients. Accumulating evidence suggests that gut microbiota via the gut-brain axis play a role in the pathogenesis of depression, thereby contributing to the antidepressant actions of certain compounds. Here we investigated the role of gut microbiota in the antidepressant effects of ketamine in lipopolysaccharide (LPS)-induced inflammation model of depression. Ketamine (10 mg/kg) significantly attenuated the increased immobility time in forced swimming test (FST), which was associated with the improvements in α-diversity, consisting of Shannon, Simpson and Chao 1 indices. In addition to α-diversity, β-diversity, such as principal coordinates analysis (PCoA), and linear discriminant analysis (LDA) coupled with effect size measurements (LEfSe), showed a differential profile after ketamine treatment. Furthermore, a total of 30 bacteria were significantly altered in the LPS + saline treated mice and LPS + ketamine treated mice. Interestingly, two bacteria, including the phylum *Actinobacteria* and the class *Coriobacteriia* were significantly correlated with the immobility time of FST. Importantly, the receiver operating characteristic (ROC) curves demonstrated that the phylum *Actinobacteria* and the class *Coriobacteriia* were potential biomarker for the antidepressant effects of ketamine in an inflammation model. These findings suggest that antidepressant effects of ketamine might be related to the regulation of abnormal composition of gut microbiota, and that the phylum *Actinobacteria* and the class *Coriobacteriia* might be potential biomarkers for ketamine's antidepressant efficacy.

1. Introduction

Depression is a significant contributor to the global burden of disease and affects people in all communities across the world (World Health Organization, 2017). Despite the introduction of several new antidepressants, two-third patients with depression respond to the first antidepressant therapy. Approximately one-third depressed patients exhibit treatment-resistant symptoms, including suicidal thoughts. Ketamine, a non-competitive *N*-methyl-D-aspartic acid receptor (NMDAR) antagonist, shows rapid-acting and sustained antidepressant effect in the treatment-resistant patients with major depressive disorder (MDD) or bipolar disorder (BD) (Berman et al., 2000; Diazgranados et al., 2010; Murrough et al., 2013; Zarate et al., 2006). Interestingly, ketamine can rapidly reduce suicidal ideation in patients with MDD (Diazgranados et al., 2010; Grunebaum et al., 2018; Murrough, 2015; Price et al., 2014). Meta-analysis demonstrated that ketamine exhibits rapid and sustained antidepressant effects and anti-suicidal ideation in treatment-resistant patients with MDD or BD (Kishimoto et al., 2016; Newport et al., 2015; Xu et al., 2016). However, the mechanisms underlying the therapeutic effects of ketamine have not yet been fully determined (Chaki, 2017; Duman et al., 2016; Hashimoto, 2016a, 2016b; Krystal et al., 2013; Monteggia and Zarate, 2015; Zanos and Gould, 2018).

Gut microbiota refers to a great number of microorganisms that coexist in the digestive tract and are an integral part of the body. Currently, it is increasingly recognized that the gut microbiota may play a critical role in human health (Sekirov et al., 2010). Human gut process 10 trillion bacteria; this could affect all aspects in a number of

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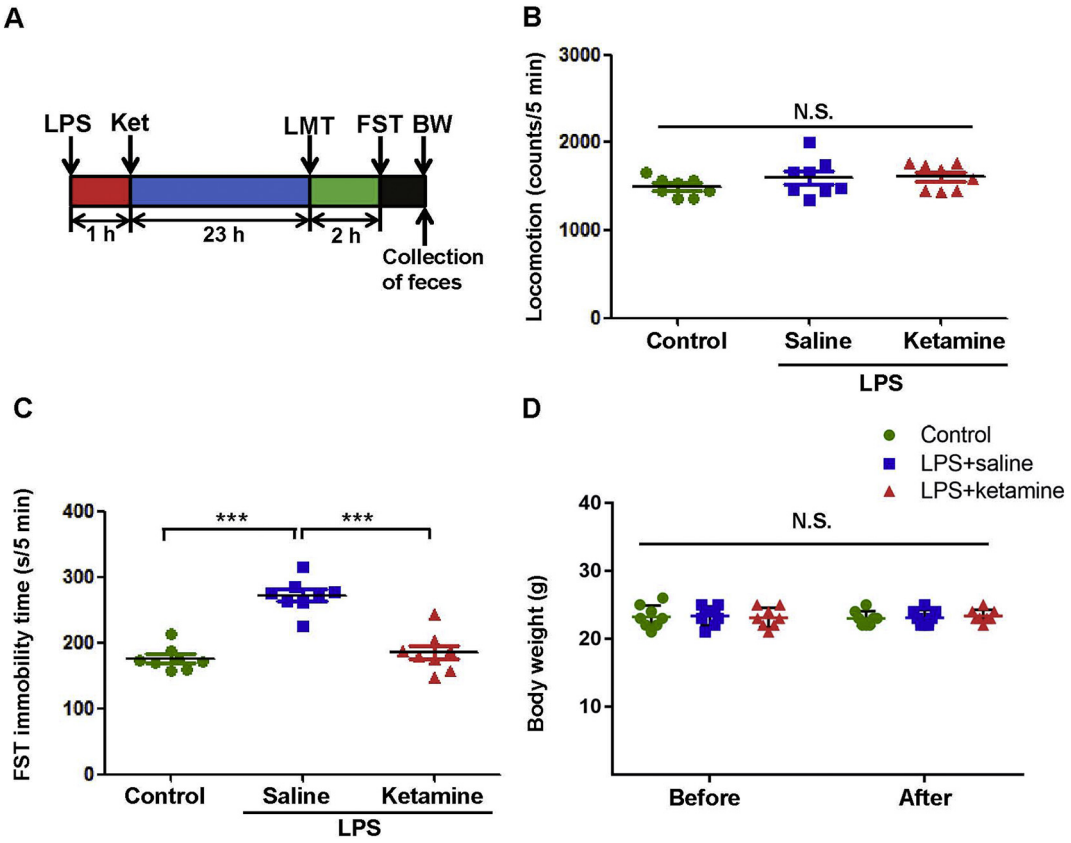

Fig. 1. Antidepressant effects of ketamine in LPS-induced depression model. (A): The schedule for the treatment, behavioral tests, and collection of fecal samples. (B): Locomotion (LMT) ($F_{2,21} = 1.282$, $P > 0.05$). (C): FST immobility time ($F_{2,21} = 36.974$, $P < 0.001$). (D): Body weight ($F_{2,21} = 0.269$, $P > 0.05$). Data are shown as means ± SEM (n = 8). ***$P < 0.001$. Ket: ketamine; LMT: locomotion; LPS: lipopolysaccharide; N.S.: not significant.

physiological functions, including body weight, digestion, infection and brain diseases (Haque and Haque, 2017; Sekirov et al., 2010). Accumulating studies suggest that the gut microbiota may contribute to the pathogenesis of depression and the antidepressant actions of certain compounds (Burokas et al., 2017; Inserra et al., 2018; Jiang et al., 2015; Wong et al., 2016; Zhang et al., 2017; Zheng et al., 2016). Recently, we reported that *Bifidobacteria* confer the resilience to the chronic social defeat stress (CSDS) model of depression (Yang et al., 2017a), suggesting the role of *Bifidobacteria* in stress resilience. Furthermore, we also reported that gut microbiota may play a role in the antidepressant effects of (*R*)-ketamine, a potent enantiomer of ketamine, in a CSDS model (Qu et al., 2017; Yang et al., 2017c). Collectively, these findings suggest that the gut microbiota might play a role in the pathogenesis of depression and in the treatment mechanisms of ketamine and its enantiomers.

The present study was undertaken to study the role of gut microbiota in the antidepressant effects of ketamine in an inflammation model of depression (Chang et al., 2018; Ma et al., 2017a; Yang et al., 2017b; Zhang et al., 2014; Zhang et al., 2018). Using 16S ribosomal RNA gene sequencing, we examined whether the gut bacterium are associated with antidepressant effects of ketamine in rodents with depression-like phenotype.

2. Materials and methods

2.1. Animals

Two-month-old male C57BL/6 mice (body weight 20–23 g) were obtained from the Animal Center of Tongji Hospital (Wuhan, China). Animals were adapted to their environmental conditions for 7 days before experiments. Animals were housed in polypropylene cages with food and water ad libitum. The room temperature was maintained at 22 ± 2 °C and a relative humidity of 60 ± 5% on a 12-h light/dark cycle (7:00 am–7:00 pm). All experimental protocols and animal handling procedures were carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health, USA (NIH Publications No. 80-23, revised in 1996). This study was approved by the Ethical Committee on Animal Experimentation of the Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology (Wuhan, China).

2.2. Drugs and treatment

Ketamine hydrochloride (Cat No.: 1707032) was purchased from Fujian Gutian Pharmaceutical Co., Ltd. (Fujian, China). On day 1, ketamine (10 mg/kg) or saline (10 ml/kg) was intraperitoneally (i.p.) administered 1 h after a single i.p. administration of lipopolysaccharide (LPS: 0.5 mg/kg, Sigma- Aldrich Co., St Louis, MO) (Ma et al., 2017b; Yang et al., 2017b; Zhang et al., 2014). On day 2, behavioral tests including locomotion (23 h after ketamine treatment) and forced swimming test (FST, 25 h after ketamine treatment) were consecutively performed. Body weight of mice was measured at baseline and immediately after behavioral tests. Subsequently, fecal samples were collected.

2.3. Locomotion

The locomotor activity was measured by an animal behavior analysis system YH-OF-M/R (Yihong Co., Ltd., Wuhan, China), the mice were placed in experimental cages (length × width × height: 1000 × 1000 × 450 mm) for 5 min. The behavioral data and trace were

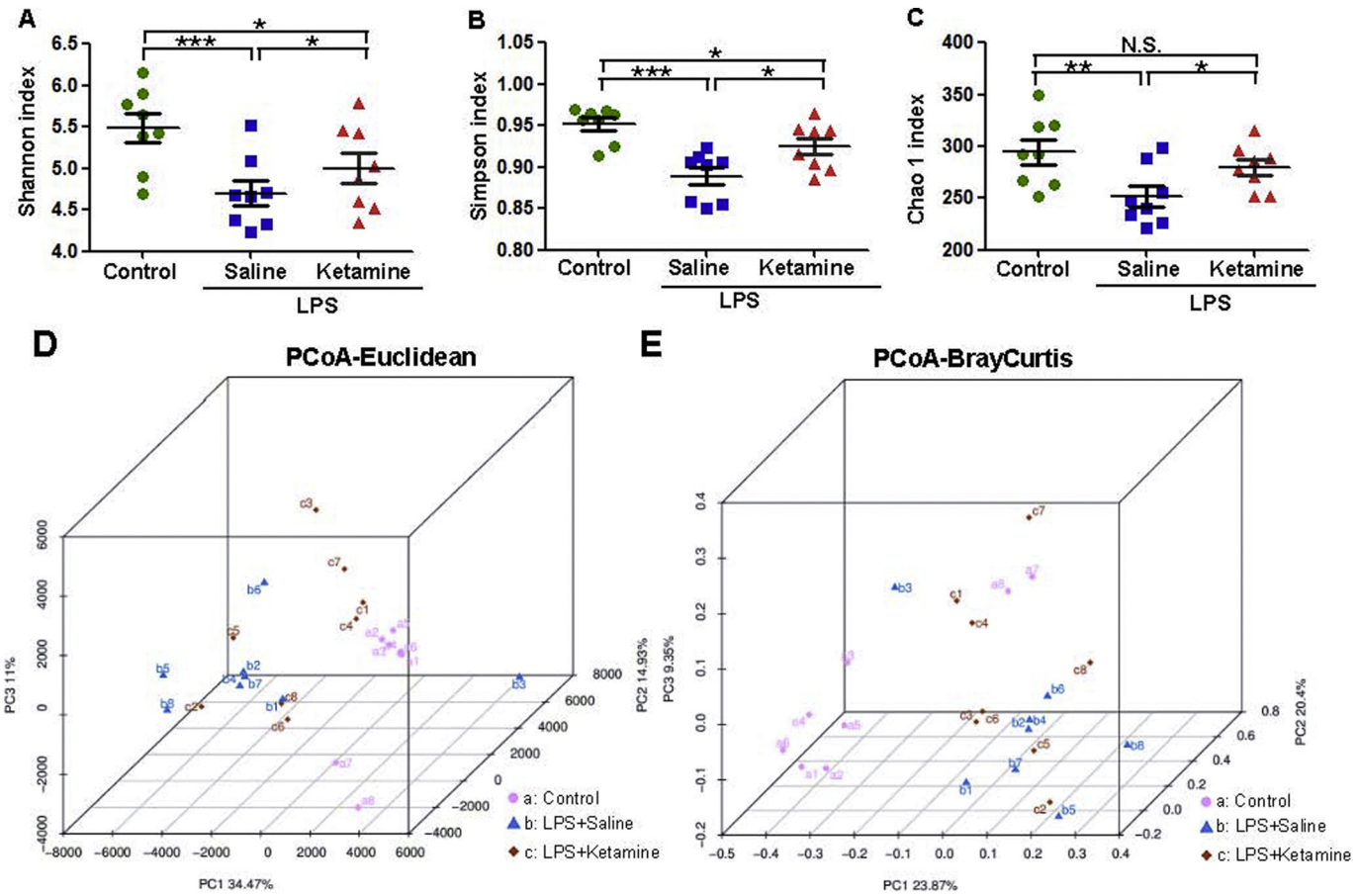

Fig. 2. Effects of ketamine on α-diversity and β-diversity in LPS-induced depression model. (A): Shannon index ($F_{2,21} = 5.673$, $P < 0.01$). (B): Simpson index ($F_{2,21} = 11.402$, $P < 0.001$). (C): Chao 1 index ($F_{2,21} = 4.829$, $P < 0.05$). (D): PCoA-Euclidean dissimilarity. (E): PCoA-BrayCurtis dissimilarity. Data are shown as means ± SEM (n = 8). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. LPS: lipopolysaccharide; N.S.: not significant.

recorded. Cages were cleaned between testing session.

2.4. Forced swimming test (FST)

The FST was tested by an automated forced-swim apparatus YH-FST (Yihong Co., Ltd., Wuhan, China). The mice were placed individually in a cylinder (diameter: 25 cm; height: 35 cm) containing 20 cm of water, maintained at 23 ± 1 °C. The immobility time for mouse was recorded for 5 min.

2.5. 16S rRNA analysis of fecal samples

The fecal samples were collected immediately after behavioral tests (Fig. 1A). Samples were placed in 1.5 ml tubes, snap-frozen on dry ice, and stored at −80 °C. The 16S rRNA analysis of the fecal samples was performed by OEbiotech Co., Ltd. (Shanghai, China). DNA extraction was performed using TIANamp stool DNA kits (Tiangen Biotechnology Company, Beijing, China). Genomic DNA was amplified in 50 μL triplicate reactions with bacterial 16S rRNA gene (V3-V5 region)-specific primers: 338F (5′-ACTCCTACGGGAGGCAGC-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). The reverse primer contained a sample barcode and both primers were connected with an Illumina sequencing adapter. PCR products were purified and the concentrations adjusted for sequencing on an Illumina Miseq PE300 system. Original sequencing reads from the sample were sorted by unique barcodes, followed by the removal of the barcode, linker, and PCR primer sequences. The resultant sequences were screened for quality and 70 or more base pairs were selected for bioinformatics analysis. All sequences were classified using the NCBI BLAST and SILVA databases. Distance calculation, operational taxonomic units (OTU) cluster, rarefaction analysis, and estimator calculation (α-diversity and β-diversity) were performed by the MOTHUR program.

2.6. Principal component analysis (PCoA)

PCoA analysis of Euclidean and BrayCurtis was performed using the rda function in the Vegan package of R ver. 3.3.2.

2.7. α-diversity analysis

α-diversity analysis including Shannon, Simpson and Chao 1 indices were determined at a sequence depth of 5000 reads per sample with 10 random iterations, using the QIIME alpha_rarefaction.py script.

2.8. Linear Discriminant Analysis Effect Size (LEfSe)

The analysis of LEfSe (Segata et al., 2011) was performed using the online version of Galaxy3. Abundances were normalized to the sum of the values in 1 million per sample and then transferred to linear discriminant analysis (LDA) via using a one-against-all strategy, and OTUs showing a score higher than 2.0 were selected.

2.9. Statistical analysis

Values presented are expressed as mean ± standard error of the mean (S.E.M.). Statistical analyses were performed using SPSS software version 17.0 (SPSS Inc., Armonk, New York, USA). Behavioral tests were analyzed by one-way analysis of variance (ANOVA) followed by

post-hoc Tukey's test. Other data were analyzed by one-way ANOVA followed by post-hoc Tukey's test, or Fisher's exact test. Correlation analysis was conducted using Pearson's product-moment coefficient. The diagnostic cut-off values, sensitivity, specificity and accuracy were determined by ROC curve analysis. *P*-values < 0.05 were considered statistically significant.

3. Results

3.1. Ketamine showed antidepressant effects in LPS-induced model

Ketamine (10 mg/kg) or saline (10 ml/kg) was administered i.p. into mice 1 h after LPS (0.5 mg/kg) administration (Fig. 1A). There was no difference in the locomotion among the three groups (Fig. 1B). In the FST, LPS significantly increased the immobility time, while ketamine significantly attenuated the increase of FST immobility time (Fig. 1C). Furthermore, there was no difference in the body weight among the three groups at baseline and after behavioral tests (Fig. 1D).

3.2. Effects of ketamine on the α-diversity and β-diversity of gut microbiota

α-diversity refers to the diversity of bacteria or species within a community or habitat. Shannon, Simpson and Chao 1 indices are commonly used to evaluate the α-diversity of microbiota. There was a significant decrease in the Shannon, Simpson and Chao1 indices in LPS-treated group, while ketamine significantly abrogated these changes (Fig. 2A–C).

PCoA is an available toll to evaluate the β-diversity of gut microbiota. PCoA plots for the three groups showed that the dots of LPS + saline treated mice (b1–b8) were not close to the dots of control mice (a1–a8) and that the dots of LPS + ketamine treated mice (c1–c8) were also not close to the dots of LPS + saline treated mice (b1–b8) (Fig. 2D and E). It is therefore likely that the composition of gut microbiota is significantly different among the three groups.

3.3. Effects of ketamine on LEfSe of gut microbiota

A total of 6 dominant bacteria were existed in control group. *Enterobacteriales* and *Gammaproteobacteria* are dominant bacteria in LPS + saline treated group. Eleven dominant bacteria are present in the LPS + ketamine treated group (Fig. 3A). A total of 16 bacteria, such as *Firmicutes*, *Clostridia*, etc., were increased in control group as compared with that of LPS + saline treated group or LPS + ketamine treated group (Fig. 3B). *Alloprevotella*, *Enterobacteriales*, *Butyricimonas*, *Romboutsia* and *Bacteroidetes* were significantly increased in LPS + saline treated group as compared with those in control group or LPS + ketamine treated group (Fig. 3B). Additionally, a total of 20 bacteria, such as *Leuconostocaceae*, *Weissella*, etc., were increased in LPS + ketamine treated group than that of control group or LPS + saline treated group (Fig. 3B).

3.4. Alterations in gut microbiota after ketamine treatment in LPS-induced model

At the phylum level, *Actinobacteria* and *Firmicutes* were decreased in LPS + saline group as compared with that of control group or LPS + ketamine group (Fig. 4A and B). *Coriobacteriia* and *Clostridia* at the class level were significantly decreased in LPS group than those in control group or LPS + ketamine group (Fig. 4C and D). At the order level, *Xanthomonadales*, *Bacillales*, *Coriobacteriales* and *Clostridiales* levels were significantly altered among the groups (Fig. 4E–H). At the family level, 9 bacteria, including *Prevotellaceae* and *Xanthomonadaceae*, were significantly altered among the groups (Fig. 4I–Q). At the genus level, a total of 12 bacteria were significantly altered after ketamine treatment in LPS-induced depression model (Fig. 4R–AC). *Lactobacillus johnsonii* at the species level were significantly increased in LPS + ketamine group (Fig. 4AD).

3.5. Correlation of FST immobility time with gut microbiota

Our analysis revealed that the phylum *Actinobacteria*, the class *Coriobacteriia*, the order *Clostridiales*, the family *Prevotellaceae* and the genus *Alloprevotella* were independently correlated with the FST immobility time (Fig. 5A–E).

3.6. Evaluation of gut microbiota for the antidepressant effects of ketamine using ROC curve analysis

The best cut-off values for the phylum *Actinobacteria*, the class *Coriobacteriia*, the order *Clostridiales*, the family *Prevotellaceae* and the genus *Alloprevotella* for the evaluation of the antidepressant effects of ketamine in LPS model were 0.000218974, 0.000330675, 0.080089621, 0.078485125 and 0.184488074, respectively. The area under the ROC curve of the phylum *Actinobacteria*, the class *Coriobacteriia*, the order *Clostridiales*, the family *Prevotellaceae* and the genus *Alloprevotella* were 0.938, 0.938, 0.578, 0.734 and 0.625, respectively (Fig. 5F). The sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of the phylum *Actinobacteria*, the class *Coriobacteriia*, the order *Clostridiales*, the family *Prevotellaceae* and the genus *Alloprevotella* for the evaluation of the antidepressant effects of ketamine in LPS model were described (Table 1).

4. Discussion

The major findings of the present study are as follows. The 16S rRNA analysis showed abnormal composition of gut microbiota in the LPS-treated mice compared to saline-treated mice, suggesting a possible role of gut microbiota in depression-like phenotype. Furthermore, ketamine (10 mg/kg) significantly attenuated increased immobility time of FST in the LPS-treated mice, consistent with previous reports of ketamine and its enantiomer (*R*)-ketamine (Chang et al., 2018; Walker et al., 2013; Yamaguchi et al., 2018; Yang et al., 2017b). Furthermore, the analysis using α-diversity, β-diversity and LEfSe suggests that the composition of gut microbiota is significantly different among the three groups (control group, LPS + saline group, LPS + ketamine group). Interestingly, the phylum *Actinobacteria*, the class *Coriobacteriia*, the order *Clostridiales*, the family *Prevotellaceae* and the genus *Alloprevotella* were independently correlated with the effects of ketamine on FST immobility time in LPS-treated mice, suggesting the role of these microbiota in the antidepressant effects of ketamine. Taken together, these results suggest that the gut microbiota may play a role in the ketamine's antidepressant actions as well as depression-like phenotype in LPS-treated mice.

The 16S rRNA analysis demonstrated abnormal composition of gut microbiota in the LPS-treated mice. In this study, we identified several microbiota which were altered in the mice with depression-like phenotypes compared to saline-treated mice. At the phylum level, *Actinobacteria* and *Firmicutes* were significantly decreased in LPS-treated mice compared with saline-treated control mice. It is reported that the gut microbiotic compositions of MDD patients and healthy controls were significantly different with MDD patients characterized by significant changes in the relative abundance of *Actinobacteria* and *Firmicutes* (Jiang et al., 2015; Zheng et al., 2017). Interestingly, the most differentially abundant bacteria taxa in female and male MDD patients belonged to the phylum *Actinobacteria* and *Bacteroidia*, suggesting that these bacteria may be sex-specific biomarkers for MDD (Chen et al., 2018). In this study, we found that ketamine significantly attenuated decreased levels of *Actinobacteria*, but not *Firmicutes*, in LPS-treated mice, suggesting a possible role of *Actinobacteria* in the antidepressant effects of ketamine. The phylum *Actinobacteria* is one of the largest taxonomic units among the major lineages currently recognized within the *Bacteria* domain (Binda et al., 2018). They have an extensive

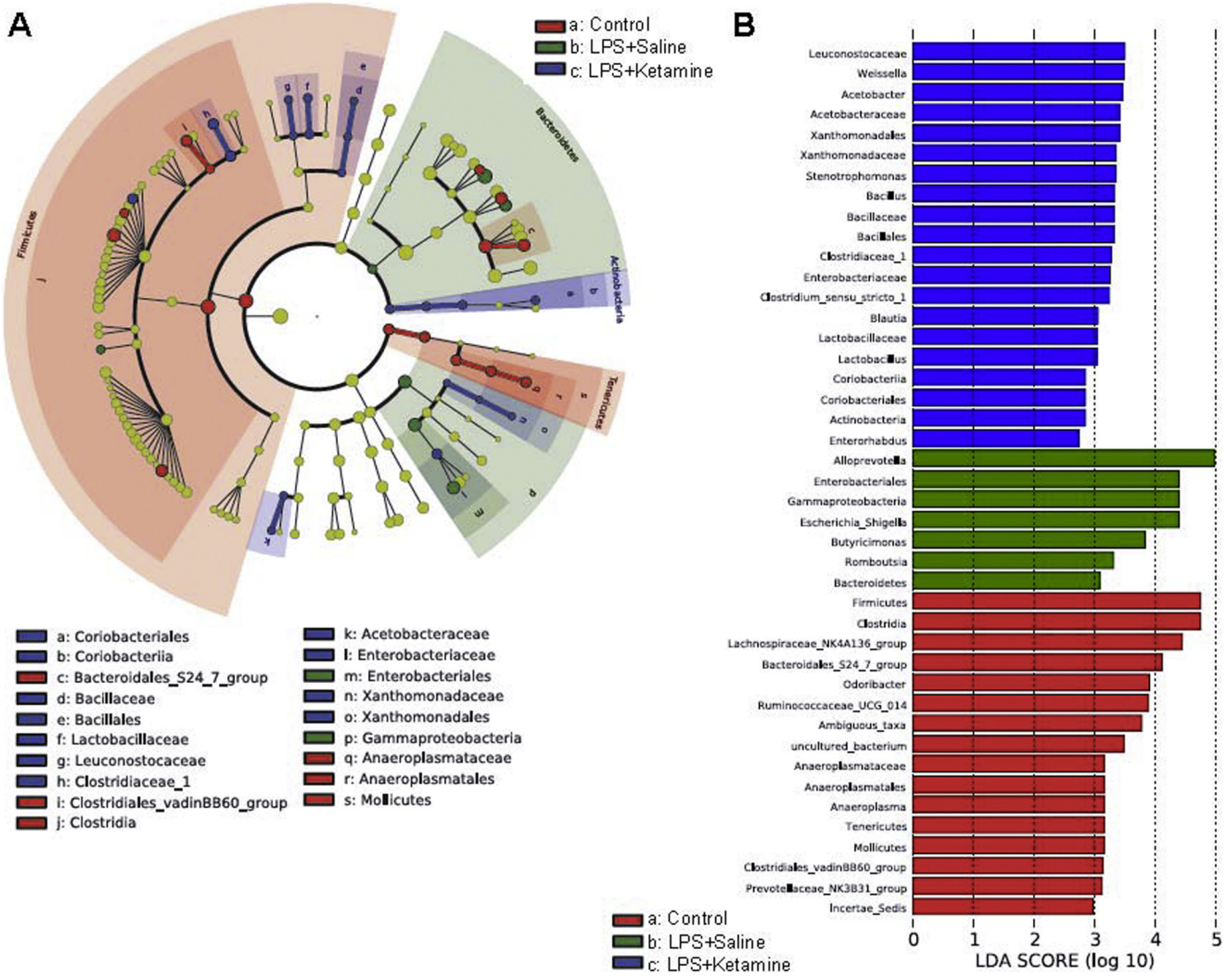

Fig. 3. Effects of ketamine on LEfSe in LPS-induced depression model. (A): A Cladogram of different abundant taxa among the groups. (B): Taxonomic groups showing LDA scores > 2.0. LEfSe: linear discriminant analysis (LDA) coupled with effect size measurements; LPS: lipopolysaccharide.

secondary metabolism and produce about two-thirds of all naturally derived antibiotics in current clinical use, as well as many anticancer, anthelmintic, and antifungal compounds (Barka et al., 2016). However, it is unclear how *Actinobacteria* play a role in the antidepressant actions of ketamine. Nonetheless, further detailed study is needed to confirm the role of *Actinobacteria* in the antidepressant effects of ketamine.

The class *Coriobacteriia* represents one of the deepest branching lineages within the phylum *Actionabcteria*, branching in the proximity of the phylum *Firmicutes*. In this study, we found that the class *Coriobacteriia* and *Clostridia* were significantly decreased in LPS + saline treated group than those in control group or LPS + ketamine group. Furthermore, the order *Clostridiales* were significantly decreased in the LPS + saline treated group than those in control group or LPS + ketamine treated group. Previously, we reported that (*R*)-ketamine, but not lanicemine, significantly attenuated the altered levels of the order *Clostridiales* in the susceptible mice after CSDS, suggesting a possible role of *Clostridiales* in the antidepressant effects of (*R*)-ketamine (Qu et al., 2017). Interestingly, patients with anorexia nervosa had reduced levels of the class *Clostridia* ($P = 0.007$) and the order *Clostridiales* ($P = 0.006$) compared to healthy control subjects, suggesting a role of these microbiota in anorexia nervosa (Kleiman et al., 2017). Therefore, it is likely that these microbiota may play a role in the ketamine's antidepressant actions as well as depression-like phenotype in rodents with inflammation although further study is needed.

In this study, we found that the family *Prevotellaceae* and the genus *Alloprevotella*, *Lachnospiraceae NK4A136*, and *Butyricimonas* were significantly increased in LPS + saline treated group than those in control group or LPS + ketamine group. It seems that abnormal composition of these microbiota may play a role in depression-like phenotype.

Very interestingly, there are significant correlations between the specific microbiota and FST immobility time in LPS-treated mice. We found significantly negative correlations between the phylum *Actinobacteria*, the class *Coriobacteriia*, the order *Clostridiales* and FST immobility time in the LPS-treated mice, suggesting that an increase in these microbiota may contribute to antidepressant-like effects of ketamine. In contrast, we also found significantly positive correlations between the family *Prevotellaceae*, the genus *Alloprevotella* and FST immobility time in the LPS-treated mice, suggesting that a decrease in these microbiota may contribute to antidepressant-like effects of ketamine. Importantly, the ROC curves demonstrated that the phylum *Actinobacteria* and the class *Coriobacteriia* were potential biomarkers for the antidepressant effects of ketamine in an inflammation model. Collectively, it is likely that normalization of these microbiota by

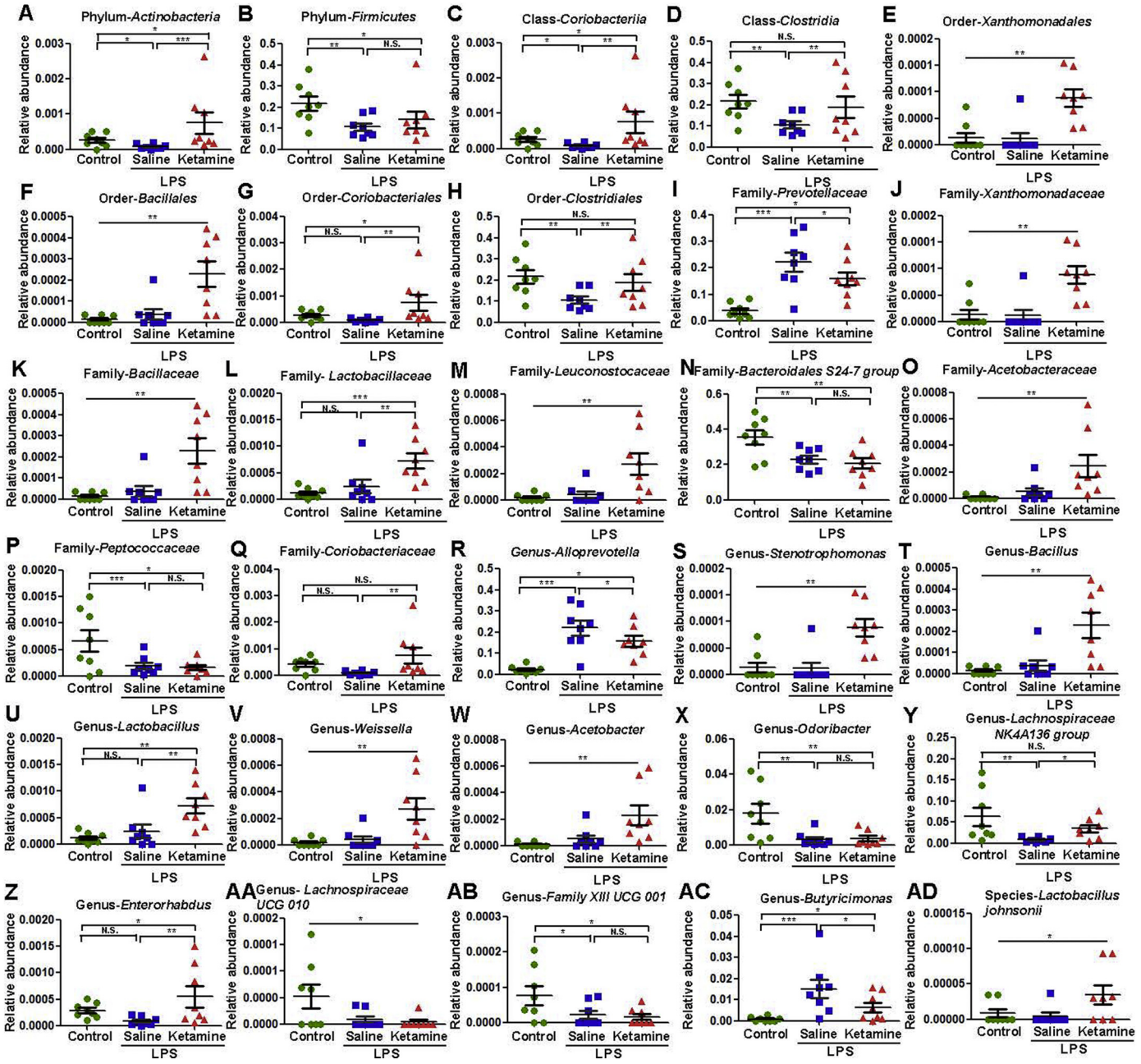

Fig. 4. Effects of ketamine on the alterations in gut bacteria in LPS-induced depression model. (A): Phylum *Actinobacteria* ($F_{2,21} = 4.421$, $P < 0.05$). (B): Phylum *Firmicutes* ($F_{2,21} = 3.746$, $P < 0.05$). (C): Class *Coriobacteriia* ($F_{2,21} = 4.265$, $P < 0.05$). (D): Class *Clostridia* ($F_{2,21} = 3.628$, $P < 0.05$). (E): Order *Xanthomonadales* (Fisher's exact test, $P < 0.01$). (F): Order *Bacillales* (Fisher's exact test, $P < 0.01$). (G): Order *Coriobacteriales* ($F_{2,21} = 3.767$, $P < 0.05$). (H): Order *Clostridiales* ($F_{2,21} = 3.987$, $P < 0.05$). (I): Family *Prevotellaceae* ($F_{2,21} = 13.369$, $P < 0.001$). (J): *Family Xanthomonadaceae* (Fisher's exact test, $P < 0.01$). (K): *Family Bacillaceae* (Fisher's exact test, $P < 0.01$). (L): Family *Lactobacillaceae* ($F_{2,21} = 8.308$, $P < 0.01$). (M): Family *Leuconostocaceae* (Fisher's exact test, $P < 0.01$). (N): Family *Bacteroidales S24–7 group* ($F_{2,21} = 6.719$, $P < 0.01$). (O) Family *Acetobacteraceae* (Fisher's exact test, $P < 0.01$). (P): Family *Peptococcaceae* ($F_{2,21} = 5.027$, $P < 0.05$). (Q): Family *Coriobacteriaceae* ($F_{2,21} = 3.627$, $P < 0.05$). (R): Genus *Alloprevotella* ($F_{2,21} = 15.742$, $P < 0.001$). (S): Genus *Stenotrophomonas* (Fisher's exact test, $P < 0.05$). (T): *Genus* Bacillus (Fisher's exact test, $P < 0.01$). (U): *Genus Lactobacillus* ($F_{2,21} = 8.308$, $P < 0.01$). (V): Genus *Weissella* (Fisher's exact test, $P < 0.05$). (W) Genus *Acetobacter* (Fisher's exact test, $P < 0.01$). (X): Genus *Odoribacter* ($F_{2,21} = 6.216$, $P < 0.01$). (Y): Genus *Lachnospiraceae NK4A136 group* ($F_{2,21} = 5.244$, $P < 0.05$). (Z): Genus *Enterorhabdus* ($F_{2,21} = 3.806$, $P < 0.05$). (AA): Genus *Lachnospiraceae UCG 010* (Fisher's exact test, $P < 0.05$). (AB): Genus *Family XIII UCG 001* ($F_{2,21} = 3.707$, $P < 0.05$). (AC): Genus *Butyricimonas* ($F_{2,21} = 3.604$, $P < 0.05$). (AD): Species *Lactobacillus johnsonii* (Fisher's exact test, $P < 0.05$). Data are shown as SEM (n = 8). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. LPS: lipopolysaccharide; N.S.: not significant.

ketamine treatment may play a role in the antidepressant effects of ketamine although further study is needed.

Zheng et al. (2016) reported that fecal microbiota transplantation of germ-free mice with 'depression microbiota' from depressed patients resulted in depression-like behaviors compared with colonization with 'healthy microbiota' from healthy control subjects. Significantly the altered phylum-level bacteria taxa of the 'inputted' human 'depression microbiota' (characterized by alterations in *Firmicutes*, *Actinobacteria* and *Bacteroidetes*) were efficiently captured in the 'output' mouse fecal communities of 'humanized' depressed mice. This study suggests that the alterations of gut microbiota and resulting induction of depression-like behaviors are transmissible (Zheng et al., 2016). Collectively, it is

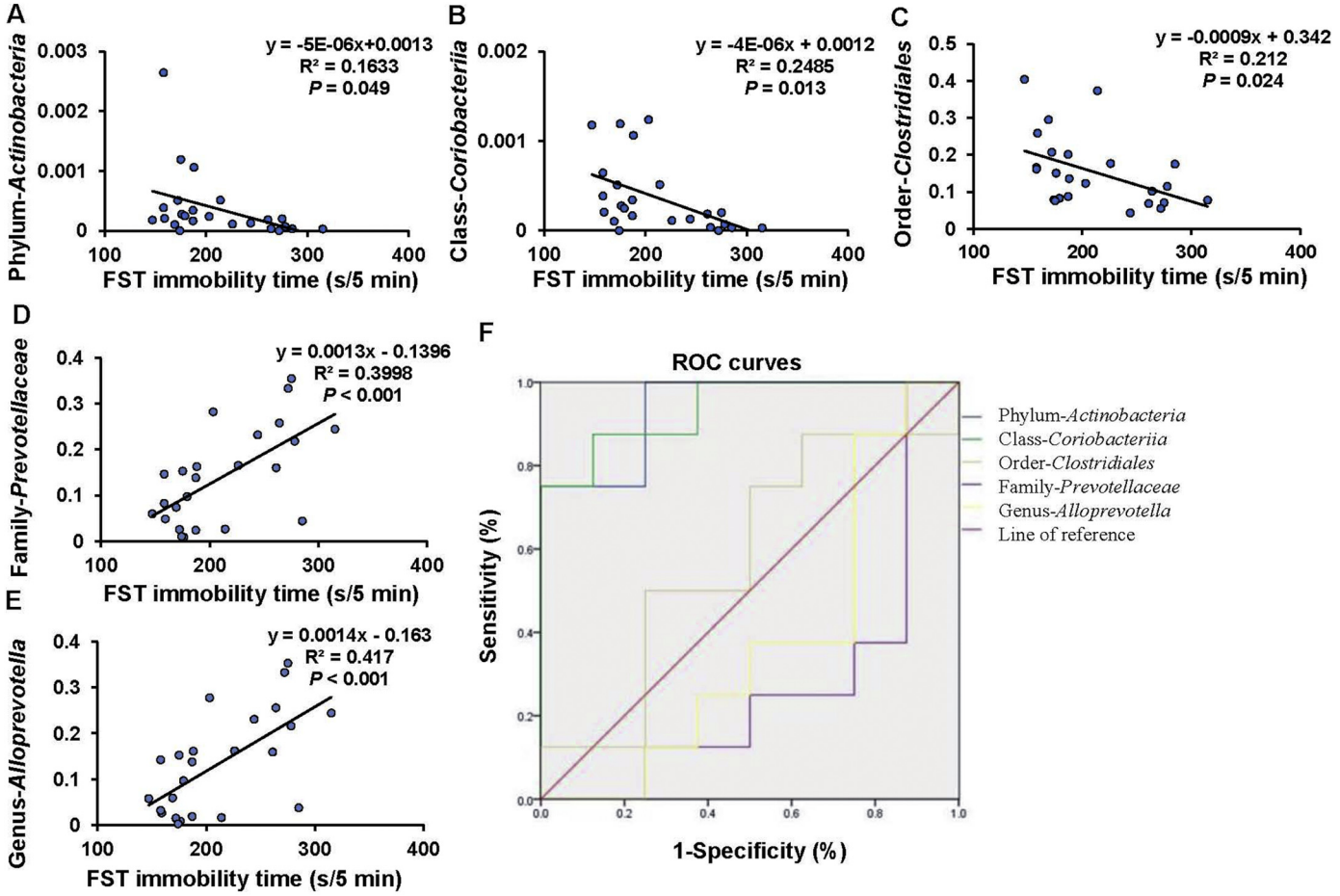

Fig. 5. Correlations between FST immobility time and gut bacteria and ROC curves. (A): Correlations between FST immobility time and the phylum *Actinobacteria* ($R^2 = 0.1633$, $P = 0.049$). (B): Correlations between FST immobility time and the class *Coriobacteriia* ($R^2 = 0.2485$, $P = 0.013$). (C): Correlations between FST immobility time and the order *Clostridiales* ($R^2 = 0.212$, $P = 0.024$). (D): Correlations between FST immobility time and the family *Prevotellaceae* ($R^2 = 0.3998$, $P < 0.001$). (E): Correlations between FST immobility time and the genus *Alloprevotella* ($R^2 = 0.417$, $P < 0.001$). (F) ROC curves of gut bacteria for evaluation of antidepressant effects of ketamine in LPS-induced model. FST: forced swimming test; LPS: lipopolysaccharide; ROC: receiver operating characteristic.

likely that normalization of these microbiota by dietary supplementation or fecal microbiota transplantation from healthy control subjects may improve depressive symptoms in depressed patients with inflammation.

Our study has some limitations. Firstly, we only observed the correlation between FST and specific gut bacteria since excessive behavioral tests may impact the levels of gut bacteria. Secondly, this study did not observe the effects of specific gut bacteria on depression behaviors, further studies are greatly needed.

In conclusion, the present study suggests that abnormal composition of gut microbiota may be associated with LPS-induced depression, and that normalization of altered composition of gut microbiota after ketamine treatment may play a role in the antidepressant effects of ketamine. Therefore, it is likely that the phylum *Actinobacteria* and the class *Coriobacteriia* might be potential therapeutic targets for inflammation-related depression.

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Conflict of interest

All the authors declared no conflict of interest.

Table 1
Evaluation of gut bacteria as potential biomarkers for the evaluation of the antidepressant effects of ketamine.

Evaluation index	Cut-off value	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Accuracy
Phylum-Actinobacteria, (n)	0.000218974	75.0% (6/8)	100.0% (8/8)	100.0% (6/6)	80.0% (8/10)	87.5% (14/16)
Class-Coriobacteriia, (n)	0.000330675	75.0% (6/8)	100.0% (8/8)	100.0% (6/6)	80.0% (8/10)	87.5% (14/16)
Order-Clostridiales, (n)	0.080089621	75.0% (6/8)	50.0% (4/8)	60.0% (6/10)	66.7% (4/6)	62.5% (10/16)
Family-Prevotellaceae, (n)	0.078485125	87.5% (7/8)	12.5% (1/8)	50.0% (7/14)	50.0% (1/2)	50.0% (8/16)
Genus-Alloprevotella, (n)	0.184488074	87.5% (7/8)	25.0% (2/8)	53.8% (7/13)	66.7% (2/3)	56.25% (9/16)

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