Characterized profiles of gut microbiota in morphine abstinence-induced depressive-like behavior

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Abstract

Morphine is the most widely used analgesic for pain management worldwide. Abstinence of morphine could lead to neuropsychiatric symptoms including depression. Gut microbiota is believed to contribute to the development of depression. However, the characters and potential role of gut microbiota in morphine abstinence-induced depression remains unclear. In the present study, we first established mice models of morphine abstinence-induced depressive behavior in mice. After dividing the mice into depressive and non-depressive groups, the gut microbiota of the mice was detected by 16S rRNA gene sequencing. The difference in the diversities and abundance of the gut microbiota were analyzed between groups. Then, the representative microbial markers that could distinguish each group were identified. In addition, gene function prediction of the operational taxonomic units (OTUs) with differential abundance between the depressive and nondepressive groups after morphine abstinence was conducted. Our results suggested that four weeks from abstinence of morphine did not change the richness of the gut microbiota. While, morphine abstinence influenced the gut microbial composition. Several specific genera of gut microbiota were identified as markers for each of the groups. Interestingly, the pathway of fatty acid metabolism was found enriched in the OUTs in the depressive group compared with the nondepressive group after morphine abstinence, by gene function prediction. Our data suggested that dysbiosis of gut microbiota was associated with morphine abstinence-induced depressive behavior, possibly by implicating the fatty acid metabolism pathway.

1. Introduction

Morphine, one of the opioids, is the most widely used analgesic for chronic pain management in the world (Rajput et al. 2021). Repeated morphine use can lead to hyperalgesia (Hu et al. 2021), antinociceptive tolerance (Dorval et al. 2021), physical and psychological dependence (Ahsan et al. 2021), as well as morphine withdrawal-induced neuropsychiatric symptoms including anxiety (Wang et al. 2016) and depression (Rauf et al. 2014), restricting the effective use of the analgesics. In addition to the high costs of morphine use in people with chronic pains, 77% of them are accompanied by a depressive state (Rauf et al. 2013), increasing morphine use-related fatality and disability. However, the mechanisms underlying morphine withdrawal-induced depression (MWD) remains elusive.

The gut is the largest gateway for the inner body to communicate with the outer substances such as the molecules of the nutrients from diets (Sharon et al. 2016). The genome of the gut microbiota is more than 150-fold than that of the human genome (Principi and Esposito 2016). Gut microbiota has been shown to contribute to the development and function of the nervous system (Sharon et al. 2016). Interestingly, in recent years, dysfunction of gut microbiota is supposed to relate to and even have causal roles in the development of many neuropsychiatric disorders including autism spectrum disorder (ASD) (Hsiao et al. 2013), schizophrenia (SCZ) (Zhu et al. 2020) and depression (Han et al. 2021; Yang et al. 2020; Zheng et al. 2016).

Gut-brain axis provides a bidirectional pathway for the communication of the gut and central nervous system (CNS) (Dalile et al. 2019). Other than the direct effect of morphine on the motor function of the gut (Penagini et al. 2004) and the composition of the gut microbiota (Hofford et al. 2021), as one of the neuroactive substances, morphine use could indirectly affect the gut microbial profiles through neuroendocrinology (Cussotto et al. 2018) or the vagal pathways (Dalile et al. 2019). Vice versa, alteration of the gut microbiota could influence the response to the drugs and the related phenotypes (Kang et al. 2017; Yang et al. 2021). Gut microbiome depletion in mice inhibited the development of antinociceptive tolerance to morphine (Kang et al. 2017) and reduced the rewarding effects of morphine in a conditioned place preference model (Hofford et al. 2021). In addition, dysbiosis of the gut microbiome could lead to depressive-like behaviors in mice through the changes of gut microbiota (Zheng et al. 2016). Whereas, the characteristics and roles of gut microbiota in the MWD remain unknown.

In the present study, the mice models with morphine abstinence-induced depressive-like behavior were first established. Following classifying the mice into depressive-like and nondepressive-like groups, the gut microbiota of the mice was detected by 16S rRNA gene sequencing. The difference in the richness and diversity of the gut microbiota between groups were compared. In addition, the representative microbial taxa that could distinguish each group were determined to better characterize the mice with different depressive-like states. The abundance of the gut microbial taxa was compared between groups to reveal the key microbiota in morphine abstinence-induced depressive-like behaviors. Finally, functional prediction of the OTUs with differential abundance between the morphine abstinence-induced depressive and nondepressive groups was performed using the KEGG database.

2. Materials And Methods

2.1 Animals and drugs

A total of 28 adult (postnatal 9 weeks) male C57BL/6J mice were purchased from the Vital River Laboratory Animal Technology Co., Ltd (Beijing, China). They were housed 4 per cage, with free access to sterile food and water. The housing room was maintained at 21 ± 2°C in temperature and 55 ± 5% in humidity, and under a 12/12-h light-dark cycle. All mice were habituated to saline injection for three days before the experiment. All the protocol of our study was approved by the Medical Ethics Committees of Xi'an Jiaotong University and Yan'an University, and in accordance with the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978).

Morphine hydrochloride was obtained from The First Pharmaceutical Factory of Shenyang (Shenyang, China). It was dissolved into 0.9% saline to a concentration of 1.0 mg/ml for the following injection. Sucrose was dissolved into sterile water to a concentration of 1.5%.

2.2 Experimental procedure

The mice were randomly assigned into two groups, with 8 mice in the control group and 20 in the morphine group. The mice in the morphine group were intraperitoneally administrated with morphine (10mg/kg, once a day) for six consecutive days. The mice in the control group were injected with equivalent saline for six days. Following four weeks of abstinence from morphine or saline, the mice were subjected to a sucrose preference test (SPT) (Fig. 1A). Briefly, 24 hours before the SPT, the mice were placed into cages individually and given free access to two bottles for 12 hours. One bottle was sterile water and the other was 1.5% sterile sucrose water solution. The position of the bottles was switched after six hours to prevent side preference. Then, all mice were deprived of water and food for 12 hours. SPT was conducted in an hour and the mice were exposed to the above two bottles, during which the side of the bottles were changed after 30 minutes. The sucrose preference was defined as 100%×sucrose intake (g) /[sucrose intake (g) + water intake (g)] in one hour.

2.3 Fecal samples collection, DNA extraction and 16S rRNA gene sequencing

Following SPT, the fecal samples were collected immediately after the mice were excreted by abdominal massage, and directly put into sterile tubes. The DNA was extracted from the fecal pellets with the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany). After quantitation, the fecal DNA was amplified by PCR with primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 805 R (5'-GACTACHVGGGTATCTAATCC-3') to amplify the V3 to V4 region of the 16S rRNA gene of the gut microbiota. The subsequent sequencing was performed on the Illumina MiSeq platform (Illumina Inc., San Diego, USA) by Genesky Biotechnologies Inc. (Shanghai, China), according to the standard procedure as described by previous studies (Zhang et al. 2021). The obtained raw reads were demultiplexed and filtered with the Mothur software. Then, the clean reads were annotated to OTUs with a similarity threshold of 97% by the UPARSE software. The OTUs were taxonomically classified at phylum, class, order, family and genus levels using the Mothur by comparing to the RDP database with a confidence threshold of 80%.

2.4 Data analysis

The SPT data were analyzed using one-way ANOVA, followed by Tukey's post hoc test with SPSS, version 22.0. The significance threshold was set at $P < 0.05$. The difference in the Chao1 index and taxa abundance among groups was analyzed by the Kruskal–Wallis rank-sum test followed by Wilcoxon pairwise comparison. A false discovery rate (FDR) or q value < 0.05 was considered significant. The β diversity was determined using analysis of similarity (ANOSIM) and permutational multivariate analysis of variance (PERMANOVA) for UniFrac distances. The linear discriminant analysis (LDA) effect size (LEfSe) method was used to detect the distinctive microbial markers for each group. The LEfSe threshold was set at LDA > 2 and $P < 0.05$. Gene function prediction of the operational taxonomic units (OTUs) with differential abundance between the Morphine abstinence-induced depressive and nondepressive groups was conducted with the KEGG database, and a *P value* < 0.05 was considered significant.

3. Results

3.1 Depressive-like behavior after morphine abstinence

After four weeks of abstinence, the morphine-treated mice were divided into two groups according to the mean (73.44%) of the SPT (within one hour) in the control group. The morphine-treated mice with a SPT > 73.44% were defined as the morphine depressive (Mor-dep) group, while mice with an SPT ≤ 73.44% were defined as the morphine non-depressive (Mor-nondep) group. The SPT in the Mor-dep group was significantly lower than that of the Control and Mor-nondep groups (Fig. 1B, P = 0.0002 and $P < 0.0001$, respectively). While, no difference in SPT was found between the control and Mor-nondep groups ($P = 0.202$). For the liquid consumption in one hour, there were no significant differences among the three groups (Fig. 1C, P = 0.257, $P = 0.077$ and $P = 0.764$ for the Mor-dep vs control, Mor-nondep vs control and Mor-dep vs Mor-nondep comparisons).

3.2 Diversities of the gut microbiota between groups

According to the Chao1 index of the α diversity of the gut microbiota, no significant difference was found in the community richness (demonstrating numbers of species) between the Mor-dep vs control (FDR = 0.195), Mor-nondep vs control (FDR = 0.203), and Mor-dep vs Mor-nondep (FDR = 0.887) comparisons (Fig. 2A). 412 OTUs were overlapped in the three groups. While, 43, 22 and 32 OTUs were found unique in the control, Mor-dep and Mor-nondep groups, respectively (Fig. 2B). For the β diversity of the gut microbiota, the principal coordinate analysis (PCoA) results showed that the three groups were separately distributed and clustered, with the Mor-dep and Mor-nondep groups clustered closer, and the control group distributed further from the other two groups (Fig. 2C, P = 0.0004).

3.3 Representative markers at the genus level for each group

To characterize each group with specific microbial markers, LDA was conducted to determine the distinctive taxa in the three groups. Our results demonstrated that several representative microbial genera were found in each group. For the control group, 34 genera including Pseudoflavonifractor (LDA = 3.428, $P < 0.001$) and Lactobacillus.s (LDA = 4.271, $P = 0.001$) etc. were more enriched. Four genera including Coprobacter (LDA = 4.155, $P = 0.002$) and Enterorhabdus (LDA = 3.616, $P = 0.007$) etc. were the representative taxa in the Mor-dep group. The Mor-nondep group was found more abundant with five genera including Parvibacter (LDA = 2.169, $P = 0.002$) and Helicobacter (LDA = 3.840, $P = 0.002$) etc. (Fig. 3). The detailed representative taxa for each group was shown in Table S1.

3.4 Abundance differences of communities of the gut microbiota between groups

We analyzed the difference of abundance in the gut microbiota between groups. Based on our outcomes, at the level of genus, the abundance of Coprobacter (q = 0.038) and Enterorhabdus (q = 0.012) was higher, and Anaerotruncus (q = 0.025) was lower in the Mor-dep group compared with the control group (Fig. 4 and Table S2). The abundance of Coprobacter (q = 0.022) was higher, and Eisenbergiella and

Anaerotruncus was lower in the Mor-nondep group than that of the control group (Fig. 4 and Table S3). However, no significant difference in the abundance of microbial genera was found between the Mor-dep and Mor-nondep groups (Table S4, all $P > 0.05$).

3.5 Function prediction of the OTUs with differential abundance between the Mor-dep and Mor-nondep groups

The gene functions of the OTUs with differential abundance between the Mor-dep and Mor-nondep groups were predicted by the KEGG database. Among the top ten most significant gene functions, the pathway of styrene degradation ($P = 0.003$), ABC transporters ($P = 0.011$), fatty acid metabolism ($P = 0.012$), transporters ($P = 0.021$), other transporters ($P = 0.023$), and transcription factors ($P = 0.027$) were found more enriched in the Mor-dep group, compared with the Mor-nondep group. The detailed gene function prediction results were shown in Fig. 5 and Table S5.

4. Discussion

Dysbiosis of gut microbiota has been shown to be related to depression (Zheng et al. 2016). MWD is a common neuropsychiatric symptom following prolonged morphine use. In the current study, we detected the gut microbial profiles in the morphine abstinence-induced depressive-like disorder. The difference in the diversity and abundance of the gut microbiota between groups were analyzed. Representative microbial genera for each group were identified. Finally, gene function prediction of the OTUs with differential abundance between the Mor-dep and Mor-nondep groups was performed.

Repeated morphine treatment was shown to decrease the motility (Girón et al. 2016) and increase the permeability (Thomaz et al. 2021) of the gut, thus may influencing the composition of the gut microbiota. Our data revealed that morphine abstinence for four weeks did not change the richness of the gut microbiota in mice. While, the composition of the microbiota altered significantly following morphine abstinence. This was consistent with previous studies indicating that intermittent or sustained morphine treatment influenced the β diversity of the gut microbiota, but not the α diversity (Lee et al. 2018; Zhang et al. 2020). In addition, according to our results, the abundance of Coprobacter increased after morphine abstinence both in the Mor-dep and Mor-nondep groups compared with the control group. Though with no significant difference in the abundance of gut microbial genera between the Mor-dep and Mor-nondep groups, the Enterorhabdus abundance was found higher in the Mor-dep group than that of the control group, but lower in the Mor-nondep group compared with the control group. This also supported a separation of the composition of the gut microbiota between the Mor-dep and Mor-nondep groups.

Representative microbial taxa can serve as potential markers for the diagnosis of diseases (Olovo et al. 2021; Tarracchini et al. 2021). We found several distinctive OTUs and gut microbial genera in the control, Mor-dep and Mor-nondep groups, respectively. These gut microbial markers might be used to distinguish different depressive states after morphine withdrawal. Meanwhile, the distinctive gut microbiota among groups demonstrates the differential alteration in the gut microbiota following morphine abstinence.

Metabolites provide a pathway for the interaction of gut microbiota and the function of CNS (Henriques et al. 2020), either directly across the blood-brain barrier (BBB) (Luo et al. 2021) or indirectly through the neuroendocrinology system (Cussotto et al. 2018). Supplementation of propionic acid, one of the short-chain fatty acids (SCFAs), could alleviate multiple sclerosis and the related immune dysfunction (Duscha et al. 2020). SCFAs intake was shown to help to improve the recovery of limb motor function after stroke in mice (Sadler et al. 2020). In our study, gene function prediction of the OTUs with differential abundance between the Mor-dep and Mor-nondep groups demonstrated that the pathway of fatty acid metabolism was enriched in the Mor-dep group compared with the Mor-nondep group. Hence, disturbance of several specific gut microbiota may be associated with morphine abstinence-induced depressive behavior through the pathway fatty acid metabolism.

To the best of our knowledge, this is the first study to demonstrate the characters of the gut microbial profiles after morphine abstinence-induced depressive behavior. However, there were several limitations in our study. First, the sample size is limited, which might restrict the identification of some gut microbiota with low abundances. Second, the resolution of the 16S rRNA gene sequencing is lower than that of the metagenome sequencing, which can determine the species of the organism. Third, only an association of dysbiosis of gut microbiota and morphine abstinence-induced depressive behavior was conducted in the present study, the functional roles of the gut microbiota in the MWD are needed to be clarified.

In summary, we observed characterized gut microbial profiles and distinctive microbial genera in mice following morphine abstinence-induced depressive-like behavior. Although no difference in the abundance of the gut microbiota was found between the Mor-dep and Mor-nondep groups, gene functions of the OTUs that with differential abundance between the above two groups included the pathways of fatty acid metabolism. Our findings suggest that there is an association between morphine abstinence-induced depressive-like behavior and dysbiosis of the gut microbiota. Further functional and mechanism research are needed to reveal the role of gut microbiota in the MWD.

Declarations

Data Availability Statement

The datasets generated during the current study are available from the corresponding author on reasonable request.

Ethical Statement

Ethics Approval and Consent to Participate

The protocol of our study was approved by the Medical Ethics Committees of Xi'an Jiaotong University and Yan'an University, and in accordance with the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978). Consent to Participate was not applicable.

Consent for Publication

Not applicable.

Availability of Data and Materials

All data and materials will be available upon reasonable request.

Competing interests

There is no conflict of interest among the authors.

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Authors' contributions

Yonghui Dang and Ruiyang Xue conceptualized and administrated this study. Jinshan Ji an Ni Yan analyzed the data and drafted the manuscript. Zhengxiang Zhang and Baoli Li contributed to the methodology. All the authors contributed and approved the final version of the publication.

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Figures

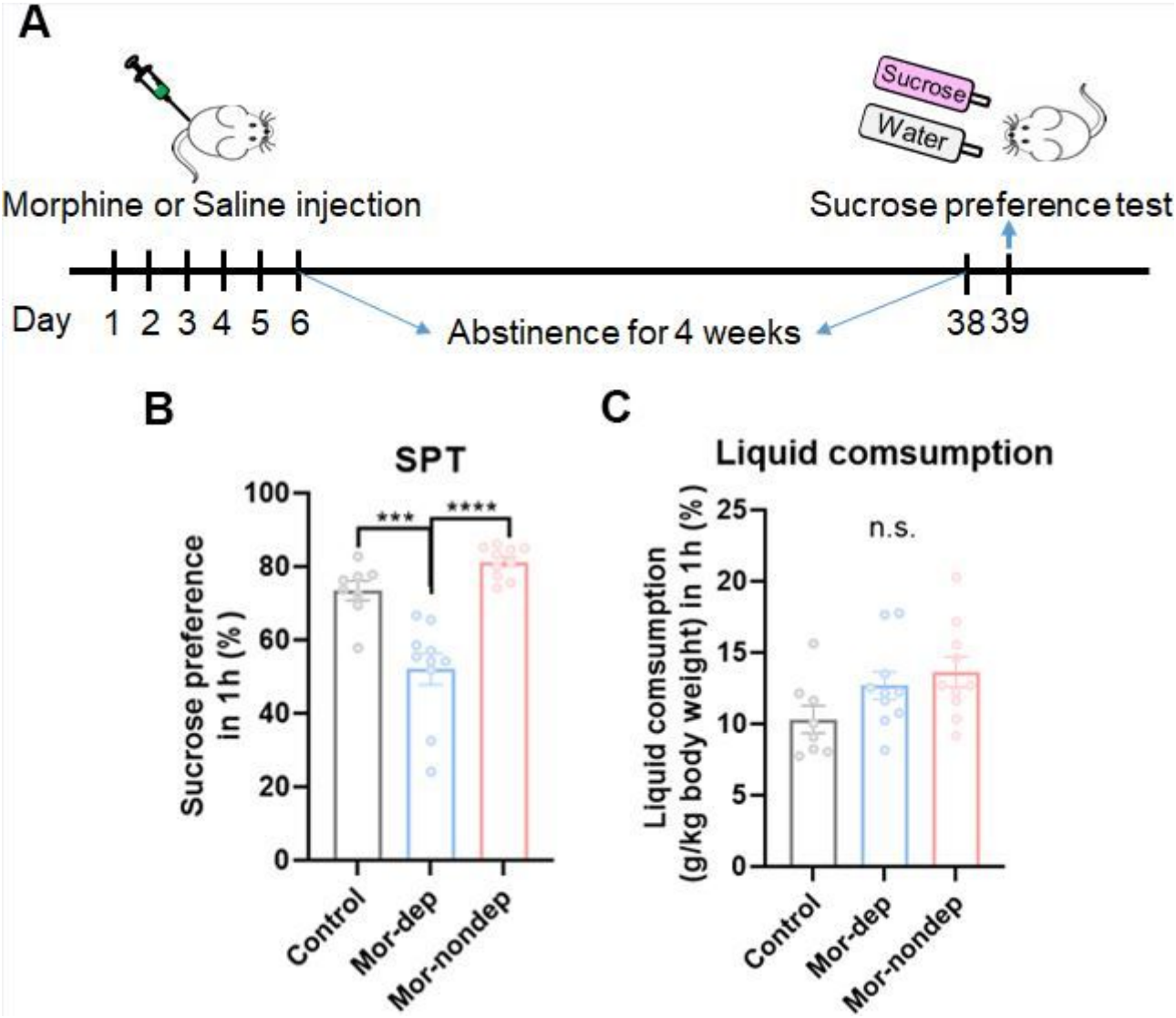

Figure 1

Procedure and results of the morphine abstinence-induced depressive behavior. A, Procedure of the SPT. B, Comparison of the SPT between groups. C, Liquid consumption of the mice in the three groups. n = 8 in the control group, and both n = 10 in the Mor-dep and Mor-nondep groups. ***$P < 0.001$, **** $P < 0.001$ between groups. SPT: sucrose preference test; Mor-dep: morphine depressive; Mor-nondep: morphine nondepressive; h, hour; n.s.: not significant.

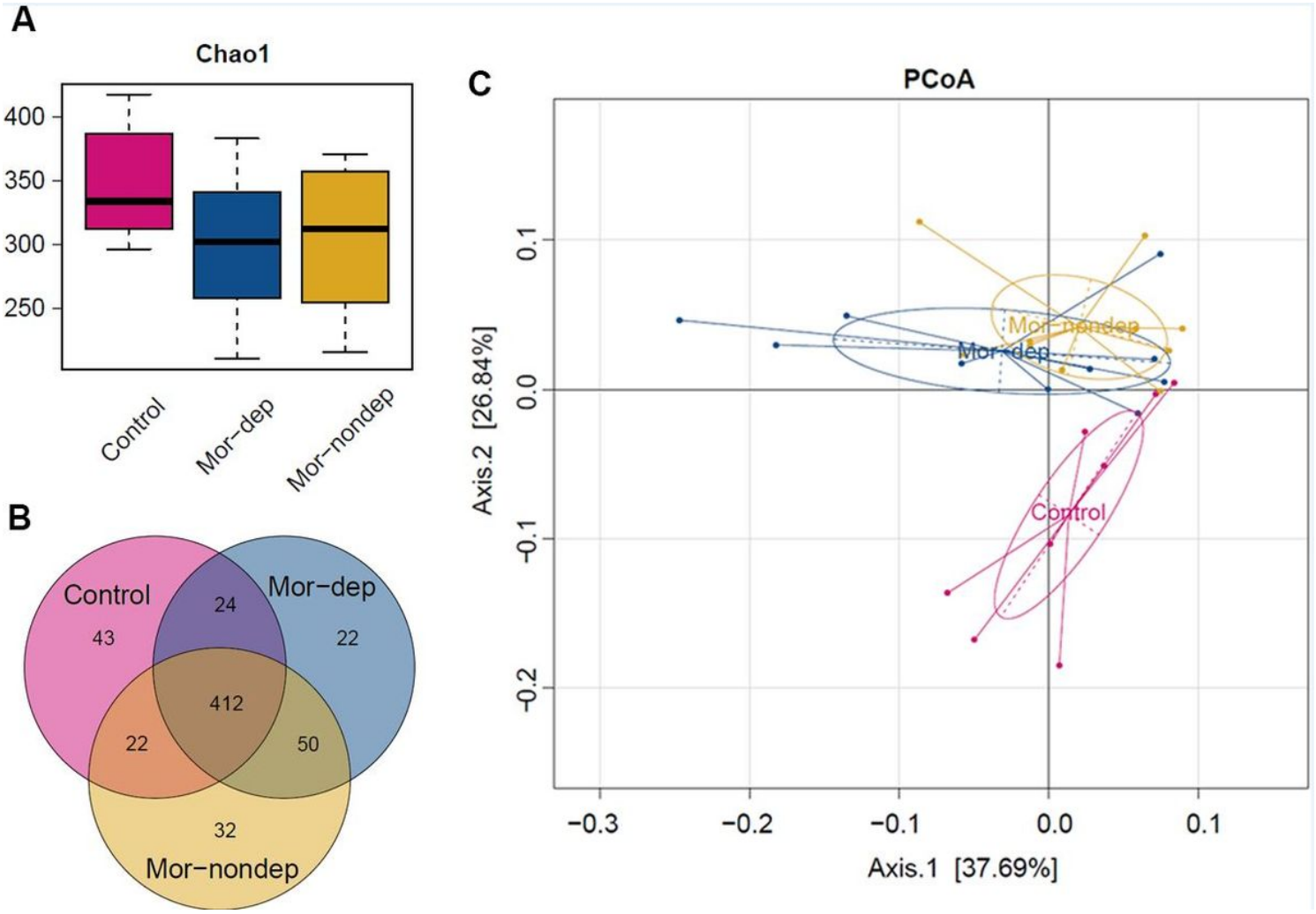

Figure 2

Diversity of the gut microbiota in different groups. A, Chao1 index of the gut microbiota in the three groups. B, Veen plot demonstrating the overlap and distinctions of the OTUs among the three groups. C, PCoA plot of the β diversity of the gut microbiota among groups. Mor-dep: morphine depressive; Mor-nondep: morphine nondepressive; PCoA: principal coordinate analysis.

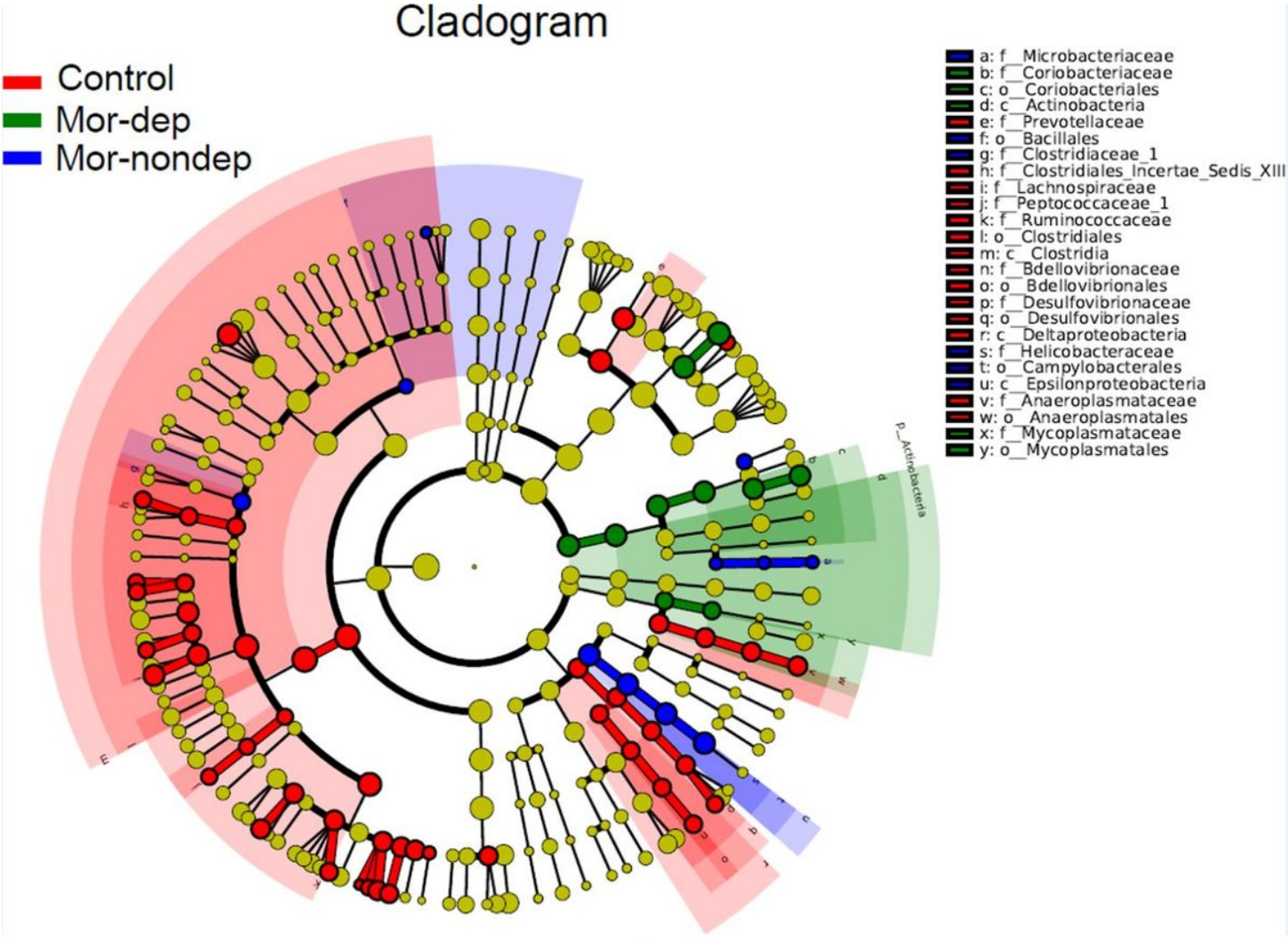

Figure 3

Makers of the gut microbiota for each group. Mor-dep: morphine depressive; Mor-nondep: morphine nondepressive.

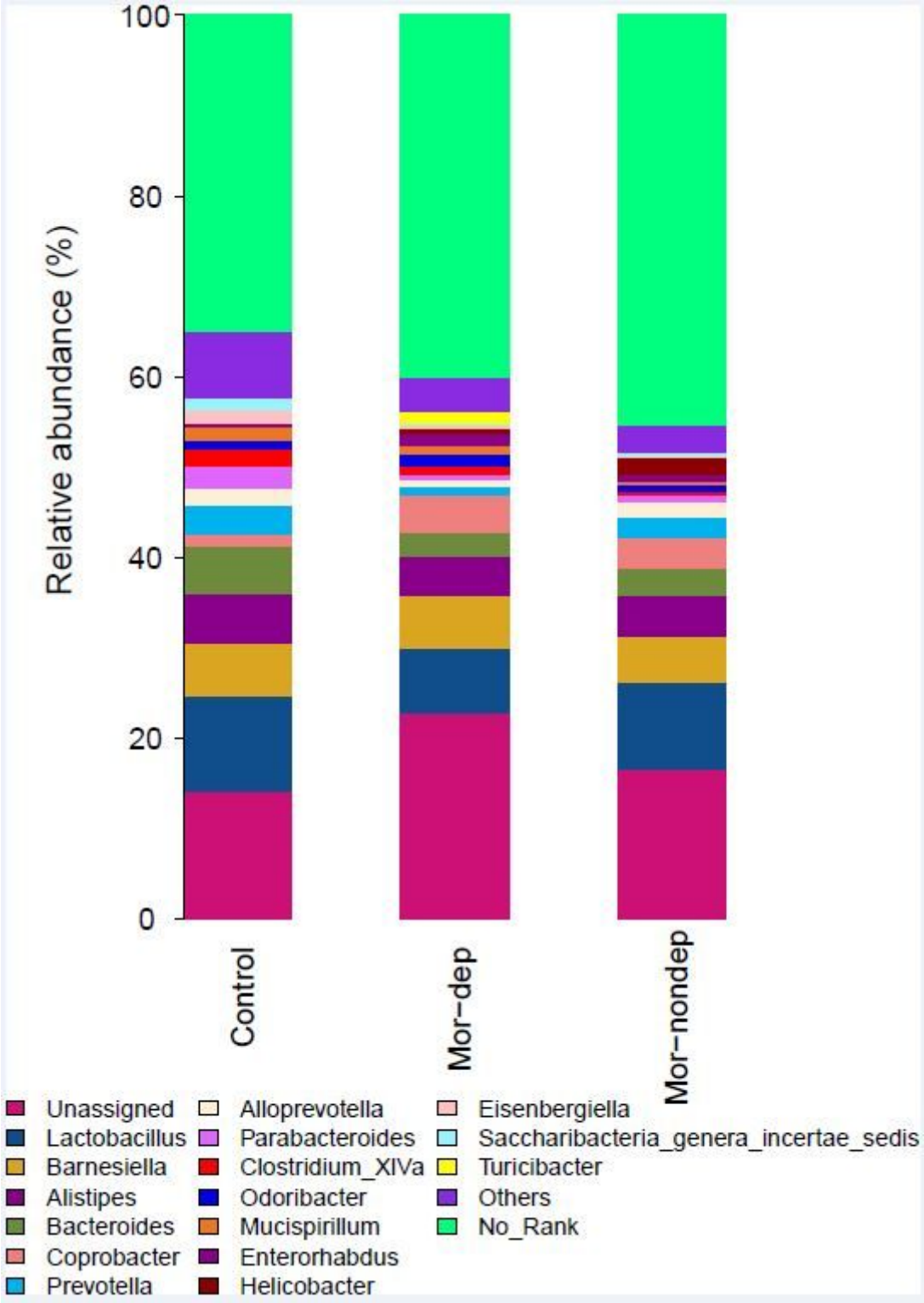

Figure 4

The abundance of the gut microbiota at the genus level. Mor-dep: morphine depressive; Mor-nondep: morphine nondepressive.

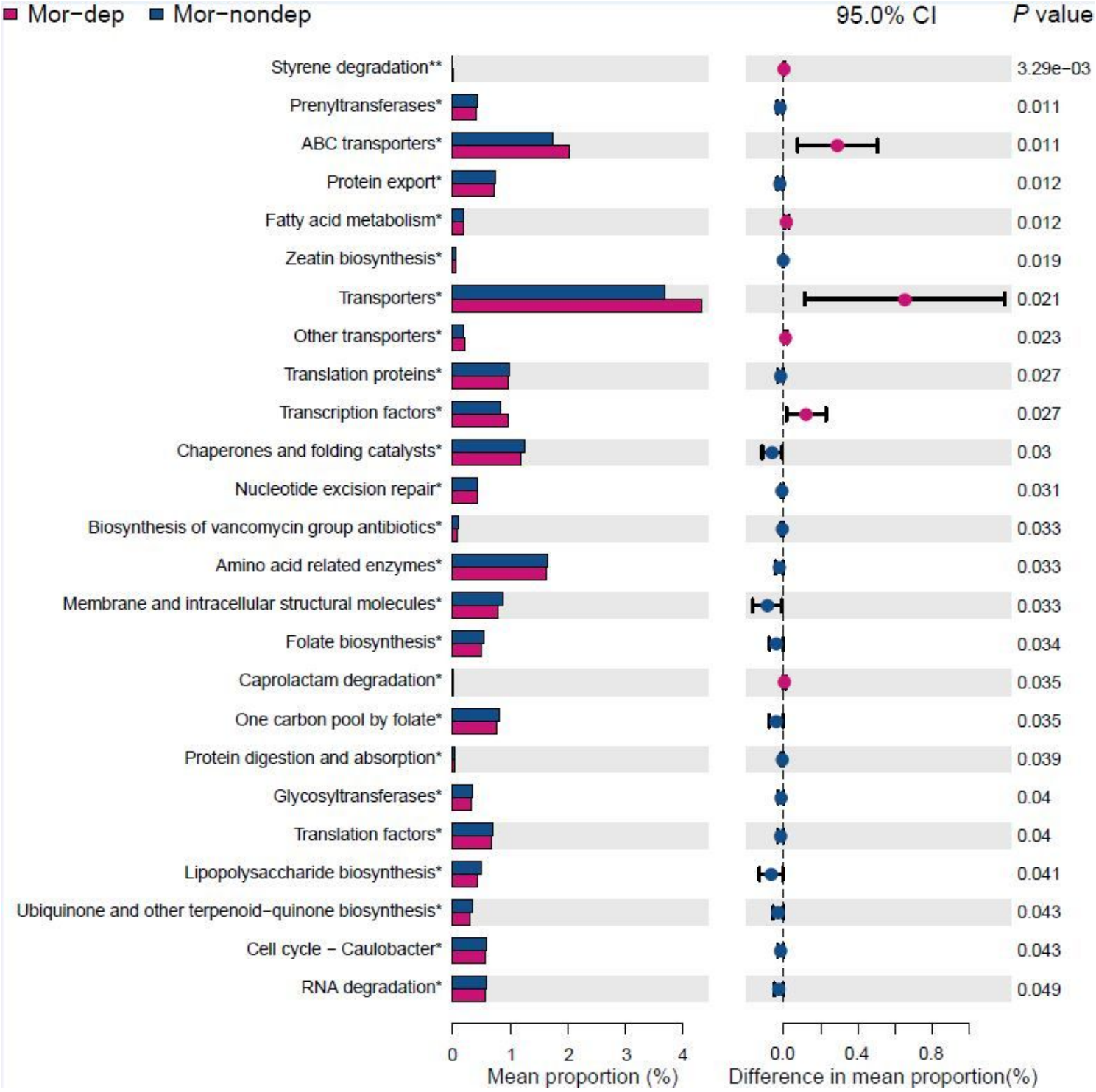

Figure 5

Functional prediction of the OTUs with differential abundance between the Mor-dep and Mor-nondep groups. Mor-dep: morphine depressive; Mor-nondep: morphine nondepressive; CI: confidence interval.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarytables.xlsx