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Exploration of microbiota targets for major depressive disorder and mood related traits



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ABSTRACT

Growing evidence suggests the link between gut microbiota and mood regulation. The current study aimed to identify microbiota targets for major depressive disorder (MDD) and mood-related traits in Taiwanese samples, while taking into account the influence of dietary patterns. We recruited 36 MDD patients and 37 healthy controls for 16S rRNA gene sequencing. We assessed nutrient content using food frequency questionnaire, and mood related phenotypes, including depressive severity, anxiety, and perceived stress. Analysis of composition of microbiomes (ANCOM) models were performed to evaluate microbiota compositions between patients and controls, while adjusted for fat intake% and sequencing platforms. We found 23 taxa (4 phyla, 7 families and 12 genera) to be associated with depression and beta diversity was differed between groups. Phylum Actinobacteria and Firmicutes were overrepresented in MDD patients. At genus level, *Bifidobacterium* (7%) and *Blautia* (8%) had relatively high abundance among MDD patients, while *Prevotella* (16%) had high abundance in controls. *Holdemania* exhibited moderate correlation with anxiety ($r = 0.65$) and perceived stress level ($r = 0.49$) mainly in MDD patients but not controls. Pathway analyses revealed that pentose phosphate and starch and sucrose metabolism processes were important pathways for depression via microbiota functions. In conclusion, our results revealed microbiota targets for depression that are independent of fat intake. It is worthwhile to conduct further studies to replicate the current findings and to integrate with biochemistry and metabolomics data to better understand the functions of identified targets.

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1. Introduction

Major depressive disorder (MDD) is a severe mental disorder, which accounts for great burden of disease worldwide (Whiteford et al., 2013). Around one-third of MDD patients suffer from repeated episodes, and a large proportion of patients do not respond to current treatment (Judd, 1997; Rush et al., 2006), which poses a substantial public health concern. Many efforts have been put in finding the underlying mechanisms for MDD to assist for designing more effective intervention and treatment strategies.

Recently, growing evidence has shown that microbiota-gut-brain axis is an important route for the bidirectional communication between the gut microbiota and the brain, for which intestinal microbiota play crucial roles in healthy brain function and affect host behaviors (Collins et al., 2012). Microbial colonization during early-life regulates hippocampal serotonergic system, and has prolonged influence on mood regulation later in life (Clarke et al., 2013). Probiotic treatments have shown beneficial effects to reduced depressive-like behaviors in rodents (Bravo et al., 2011; Desbonnet et al., 2010). The causal effects of microbiota on depression were further strengthened by using germ-free mice or microbiota deficient mice and fecal transplantation of microbiota from depressive patients (Kelly et al., 2016; Zheng et al., 2016). Using germ-free or microbiota deficient animal models allows researchers to study effects of specific taxa on mood regulation. Moreover, microbiota transplantation studies serve as a great tool to explore the overall effects of human microbiota on depressive-like behaviors. Findings from previous animal studies have enhanced our knowledge about the influences of microbiota on depression (Cryan and Dinan, 2012). Nevertheless, it is known that mice microbiota compositions are quite different from those in humans, and many other factors might influence on human microbiota, such as diet, genetic factors, and drugs (Nguyen et al., 2015). It is thus fundamental to examine such relationships in different human populations for future translational research.

There are a few studies that addressed this question by exploring differences in human microbiota compositions between individuals with depressive mood and healthy controls. Studies are often conducted to compare dozens of MDD cases and controls. For instance, Naseribafrouei et al. (2014) reported that MDD patients had higher proportion of the order Bacteroidales and the genus *Alistipes*, and lower proportion of the family Lachnospiraceae and the genus *Oscillibacter* than healthy controls in a Norway sample. Another three studies conducted in Chinese population discovered microbiota differences in family Lachnospiraceae, which is a target consistently reported across studies (Jiang et al., 2015; Liu et al., 2016; Zheng et al., 2016). However, none of the microbiota target in the genus or species levels are replicated across studies, which can be attributable to different study designs, analysis strategies, and subject characteristics. In particular, some of the important confounding factors are not well controlled in previous studies and might bias the reported findings. For instance, dietary intake is known to have substantial impacts on human microbiota compositions (Zhang et al., 2010). However, dietary factors were not measured and thus, not taken into account in previous microbiota studies. It is essential to consider important confounders in searching for relevant microbiota targets for MDD. In addition, it is desirable to reliably identify microbiota targets at a lower level, such as genus or species level, to assist to design follow-up experiments to explore their biological functions and interaction mechanisms with the host.

On the other hand, patients with MDD possess substantial clinical heterogeneity, with varied symptom severity, comorbidity patterns, or reactivity to stress across individuals (Burke et al., 2005; Sartorius et al., 1996). How the microbiota targets for MDD play roles in mood related clinical characteristics is largely unknown. Most of the previous evidence of emotional regulation by microbiota comes from animal studies. For instance, comparing with the specific pathogen free mice,

GF mice revealed higher corticosterone and adrenocorticotropic hormone levels in response to stress stimuli (Sudo et al., 2004). In addition, mice exposed to chronic social stress showed immediate changes in decreasing genus *Bacteroides* and increasing genus *Clostridium* (Bailey et al., 2011). Administration of *Bifidobacteria* and *Lactobacillus* formulated probiotic was reported to reduce anxiety-like behaviors in rats model (Messaoudi et al., 2011). In human, relatively limited studies investigated the associations between microbiota targets and mood related traits. One study in Chinese population ever reported that the genus *Faecalibacterium* is negatively correlated with depressive severity (Jiang et al., 2015), but more targets await to be discovered for relevant traits. Because intestinal bacteria are involved in regulating a wide range of metabolic homeostasis, and emotional and behavioral phenotypes in human body (Alcock et al., 2014; Tremaroli and Bäckhed, 2012), it is our interest to evaluate the associations between the identified microbiota targets and mood related traits, such as anxiety and stress.

Last but not the least, to explore the functional interpretation for the different microbiota compositions between MDD patients and healthy controls can assist for a better understanding of the biological pathways involved in the gut-brain axis for MDD. A recent study tested the metabolomic profiles between mice transplanting with fecal samples obtained from MDD patients or healthy controls (Zheng et al., 2016). The authors reported that carbohydrate and amino acid metabolisms are up-regulated among mice harboring depression microbiota. Comparable studies in human are rarely evaluated so that the biological pathways of microbiota composition for MDD require further research (Chen et al., 2018b). Previously, studying bacteria properties and functions mainly relies on successful cultures of specific bacteria, but the majority of bacteria cannot be cultured. The functional predictive algorithm based on genetic inferences provides us a new venue to explore the functions of microbiota involvement in MDD (Langille et al., 2013).

In the current study, we aimed to first conduct a case-control study in Taiwanese population to identify microbiota targets for MDD, while taking important diet variables into account. Secondly, we investigated whether specific microbiota targets of MDD are correlated with a number of relevant mood related clinical variables, such as depressive severity, anxiety, and perceived stress. Lastly, we employed pathway analysis to provide the functional interpretations for microbiota compositions of MDD.

2. Materials and methods

2.1. Subject recruitment

Patients who diagnosed with MDD according to the criteria of Diagnostic and Statistical Manual of Mental Disorders fifth version (DSM-5) are consecutively referred by psychiatrists in several central and regional hospitals in Taipei. Exclusion criteria included patients with mental retardation, schizophrenia, schizoaffective disorder, or are substance induced secondary MDD. Controls are collected from community in the same catchment area without past diagnosis of major psychiatric disorders, such as anxiety disorder, mood disorder, schizophrenia, mental retardation, and substance use disorder. The age range of cases and controls were from 20 to 65 years old. Considering of many factors might influence microbiota composition, we excluded subjects who used antibiotic, probiotic, prebiotic, symbiotic, or with known active bacterial, fungal, or viral infections, gastrointestinal surgery in the previous two months, according to the exclusion criteria used in previous studies (Jiang et al., 2015). In total, our samples consisted of 36 MDD patients and 37 controls. The present study was approved by institutional review board of all participated hospitals. Written informed consent was obtained from every participant and had been fully explained.

2.2. Mood assessment

Subjects were interviewed with modified Chinese version of Schedule for Affective Disorders and Schizophrenia-Lifetime (SADS-L) to assess demographic, physical characteristics and preliminary determination of the psychiatry disorders. Details of the SADS-L please referred to Huang et al. (2004). To obtain levels of symptom severity, we used Beck Depression Inventory (BDI) which assessed recent depressive severity within 2 weeks. Each item was scored from 0 to 3. The cutoff of 13, 19, and 28 were set to indicate minimal, mild, moderate, and severe depression. To further assess anxiety levels among individuals, we used Beck Anxiety Inventory (BAI) to measure anxiety level within 1 week, with each item scored ranging from 0 to 3. Score cutoff of 9, 16 and 29 were used to indicate minimal, mild, moderate and severe anxiety. Finally, 10 items Perceived Stress Scale (PSS) was used to measure stress level. Every item was scored from 0 to 4. Scores of 0–13, 14–26 or 27–40 represent low, moderate and high perceived stress, respectively.

2.3. Dietary assessment

Considering the substantial influence of dietary intake on microbiota composition, we assessed dietary variables using a semi-quantitative food-frequency questionnaire (FFQ) with 31 items, which contain information on six basic food groups, sugar and dietary supplements consumption. This is a shorter version modified from a previously developed Taiwanese FFQ (Lee et al., 2006), with good reliability and validity. Details of the FFQ please referred to Chou et al. (2011). Subjects were asked about the average frequency of consumed food categories based on a standardized unit or portion size of each food during the previous year. We can then obtain the consumption information of carbohydrates, proteins, fat intake and various types of fatty acids. The saturated fatty acids were further classified as short, medium and long-chain fatty acids according to carbon numbers (Huang et al., 2011).

2.4. Bio-sample collection and stool DNA extraction

Approximately 1g of the stool samples were collected within 2 weeks of the interview and severity assessments. All stool samples were delivered in 4 °C and stored in –80 °C refrigerator immediately after transferring to 2c.c eppendorf. Microbiota DNA was extracted from stool using either QIAamp DNA Stool Mini Kit (QIAGEN Inc., USA) or phenol–chloroform extraction method. All lysis, separation of impurities and purification procedures were followed the manufacturer protocol of QIAamp DNA Stool Mini Kit. While using phenol–chloroform extraction method, we followed the extraction protocol from Nakayama et al. (2015). Briefly, all samples were diluted twice with PBS. Afterwards, glass beads and saturated phenol were applied to the samples and vortexed via FastPrep FP120 (Bio 101) at a speed of 5.0 m/sec for 30-sec. Following steps required adding phenol/chloroform/isoamyl alcohol (25:24:1; v/v) to supernatants and went to second shake by FastPrep FP120 at a speed of 4.0 m/sec for 45sec. 3 M sodium acetate (pH 5.2) and subsequent 100% isopropanol were then added to the supernatants and washed by 70% ethanol. The pellets were air dried and suspended in TE buffer until use. All the DNA samples were evaluated for the quality via NanoDrop Microvolume Spectrophotometers.

2.5. 16S ribosomal RNA gene sequencing

In order to obtain the microbiota composition, 16S ribosomal RNA (16S rRNA) gene sequencing was conducted. In total, 53 (27 MDDs and 26 controls) and 20 samples (9 MDDs and 11 controls) were sequenced via Illumina MiSeq and MiniSeq platforms, respectively. We mixed cases and controls within each platform to reduce sequencing bias between cases and controls caused by batch effect. In Illumina MiSeq platform, forward primer: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3' and reverse primer: 5'-GTCTCTGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3' were used for amplifying V3–V4 region, while in MiniSeq platform, forward primer: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGCCAGCMGCCGCGGTAA-3' and reverse primer: 5'-GTCTCTGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTWTCTAAT-3' were used for V4 region amplification. The sample reads from Illumina MiSeq were ranged from 103,228–330,268 (median = 210,298), and obtained 7524 operational taxonomic unit (OTUs), while the reads from MiniSeq were ranged from 81,749 to 1,294,044 (median = 928,601), with 6598 OTUs.

2.6. Statistical analysis

For analyzing sequencing data, we applied PEAR (version 0.9.8) to merge the paired-end reads, while considering the sequences match of reads for at least 5 bases overlapped (Zhang et al., 2014). In the subsequent taxonomy classification procedures, we used QIIME (version 1.9.1) to conduct the sequence quality filtering. The pipeline follows default parameters in QIIME, including: (1) maximum 3 consecutive low quality base call were allowed before truncating the reads, (2) at least 75% of consecutive high quality base calls were included in a read, (3) no ambiguous character allowed in the sequence, while setting Phred qualities below 20 as low quality threshold. After filtering, we clustered the sequences with 97% similarity of OTU from Greengenes taxonomic database (May 2013 version). After completing the OTU table, the taxonomies were summarized into phylum to genus levels. In total, 129 out of 611 taxa that have abundance greater than 0.01% were reserved for analysis. Four different alpha diversity (Shannon Index, Chao 1, Observed OTUs, PD Whole Tree) and two beta diversity (Unweighted and Weighted UniFrac) indices were calculated via QIIME. We examined global microbiota composition differences between MDD patients and controls using permutational analysis of variance (PERMANOVA). Moreover, we applied analysis of composition of microbiomes (ANCOM) to analyze each individual taxa abundance between MDD cases and controls (Mandal et al., 2015). The ANCOM was developed for analyzing 16S rRNA gene sequencing data, which makes no distributional assumptions, allows for adjustment of covariates, and was reported to reduce false positive rate while increase statistical power. We set $\alpha = 0.05$ at 60% of comparisons as the threshold for rejecting null-hypothesis using recommended taxa-wise multiple correction as the criteria to report significant results.

Dietary intake of major macro-nutrients was estimated via FFQ, including carbohydrates, protein, and fat. We found very high correlations among them in our samples (please see Supplementary Table 1); the absolute value of correlation coefficients were ranged from 0.8 to 0.97. In addition, prior studies reported significant associations between fat intake and changes in microbiota compositions (Cani et al., 2007; Kim et al., 2012). Therefore, we selected fat intake% as the relevant dietary variable. Thus, two covariates were put into the ANCOM models for adjustment, including sequencing platform (Miniseq or Miseq) and fat intake. Additionally, we analyzed functional pathways by Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUST, version 1.1.2) (Langille et al., 2013) to explore potential functional properties of the identified microbiota for depression.

After the identification of microbiota targets for MDD, we additionally evaluated the relationships between abundance of the targeted microbiota and clinical features, including BDI, BAI and PSS scores. We analyzed the associations by overall samples, MDD patients, and controls to explore whether there are different effects by subgroups. Because many taxa showed skewed distributions, we used Spearman's rank correlation for analysis. In presenting the correlation plots, for taxa that have extreme distribution (absolute value of skewness or kurtosis greater than 1), the taxa abundance was log-transformed before drawing the plots. All these analyses were performed by

Table 1
Demographic, clinical characteristics and dietary patterns in MDD patients and healthy controls.

| Variable | MDD case (n = 36) | Control (n = 37) | P-value |
|-------------------------------------------|----------------------|-------------------------|----------|
| Demographic characteristics | | | |
| Age, means (SD) | 45.83 (14.08) | 41.19 (12.73) | 0.144 |
| Education year, means (SD) | 13.78 (3.20) | 15.82 (2.17) | 0.003* |
| BMI, means (SD) | 22.80 (4.21) | 23.95 (3.92) | 0.232 |
| Female, No. (%) | 28 (82.35) | 23 (62.16) | 0.059 |
| Married, No. (%) | 20 (55.56) | 17 (50.00) ^a | 0.642 |
| Employed, No. (%) | 24 (66.67) | 36 (97.30) | 0.002* |
| Smoking, No. (%) | 7 (19.44) | 1 (2.70) | 0.056 |
| Clinical characteristics | | | |
| Antidepressant use, No. (%) | 31 (86.11) | 0 (0) | < 0.001* |
| Number of episodes, mean (SD) | 7.28 (8.90) | NA | NA |
| Illness duration, mean (SD) | 11.53 (9.29) | NA | NA |
| BDI score, means (SD) | 19.18 (12.47) | 4.54 (4.85) | < 0.001* |
| PSS score, means (SD) | 20.92 (5.20) | 15.00 (4.70) | < 0.001* |
| BAI score, means (SD) | 10.64 (7.57) | 3.14 (3.90) | < 0.001* |
| Dietary characteristics | | | |
| Total Energy (kcal), means (SD) | 1934.21 (885.13) | 1760.95 (829.00) | 0.391 |
| Crude fiber (g), means (SD) | 4.74 (2.88) | 4.06 (2.51) | 0.281 |
| Dietary fiber (g), means (SD) | 14.83 (9.80) | 12.56 (8.16) | 0.287 |
| Protein intake %, means (SD) | 14.55 (4.06) | 15.85 (3.46) | 0.145 |
| Carbohydrates intake %, means (SD) | 63.19 (9.21) | 57.86 (10.57) | 0.024* |
| Fat intake %, means (SD) | 21.74 (6.12) | 25.91 (7.81) | 0.013* |
| Short chain fatty acid (mg), median (SD) | 85.39 (127.31) | 193.49 (188.85) | 0.003* |
| Medium chain fatty acid (mg), median (SD) | 528.78 (456.55) | 834.54 (554.40) | 0.032* |
| Long chain fatty acid (mg), median (SD) | 13543.10 (5558.41) | 15255.33 (5776.48) | 0.108 |

*P-value < 0.05.

BDI, Beck Depression Inventory; BAI, Beck Anxiety Inventory; PSS, Perceived Stress Scale.

NA = Not applicable.

^a Three subjects had missing data in marriage status.

R (version 3.4.0).

3. Results

3.1. Demographic characteristics

Demographic characteristics are displayed in Table 1. Controls had more education years (15.82 years) and higher proportion to be currently employed (97.3%) than MDD patients (13.78 years, 66.7%). In terms of clinical characteristics, MDD patients exhibited severer depression and anxiety, with average BDI and BAI scores of 19.18 and 10.64 compared to 4.54 (BDI) and 3.14 (BAI) in controls. Similarly, MDD patients had higher PSS score than that in controls (p-value < 0.001). In addition, 86% of the MDD patients were treated with antidepressant while no controls were taken antidepressant. For dietary patterns, our results demonstrated that protein consumptions did not differ in the two groups. MDD patients had significantly higher carbohydrate intake (63% in MDD and 58% in controls) and lower fat intake (22%) comparing to controls (26%), in particular at the saturated short-chain fatty acid (85.39 mg in MDD patients vs. 193.49 mg in controls) and medium chain fatty acids categories (528.78 mg in MDD patients vs. 834.54 mg in controls).

3.2. Microbiota composition between MDD patients and controls

Overall speaking, there were no significant differences in indices of alpha diversity between MDD patients and healthy controls. The

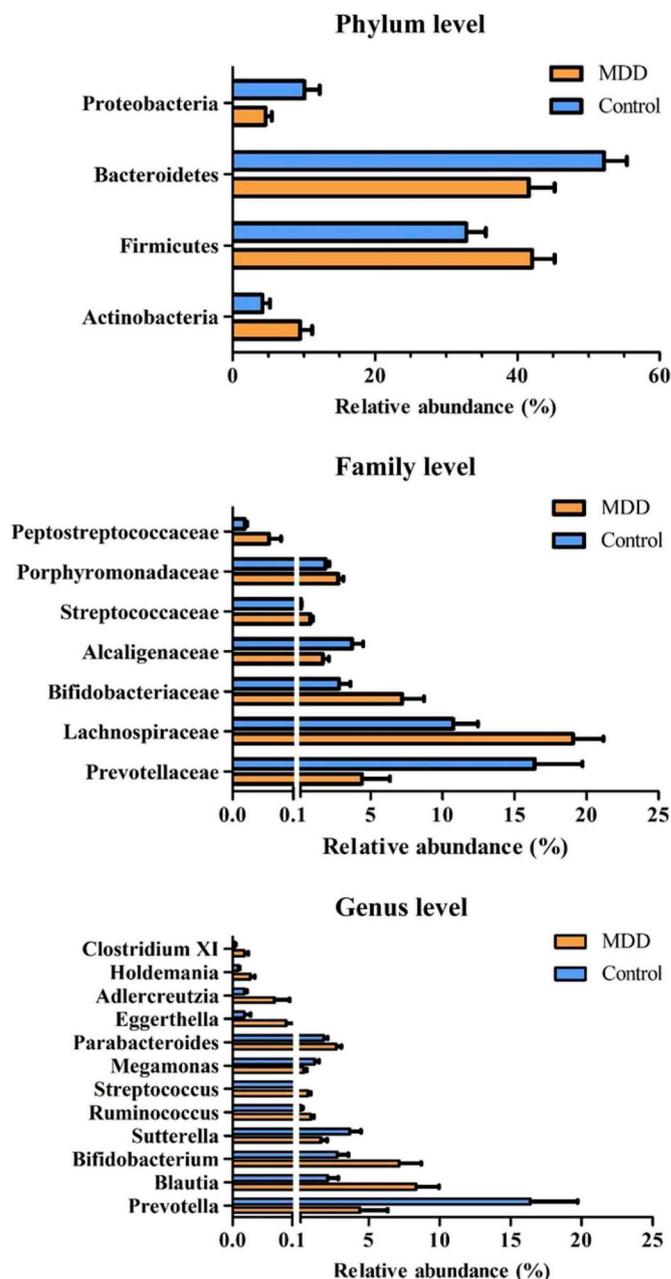


Fig. 1. Relative abundance of microbiota targets in MDD patients and healthy controls.

Shannon index and indices of beta diversity are displayed in Supplementary Figs. 1–3 for all samples. On the other hand, PERMANOVA revealed significant composition differences between the two groups (p-value = 0.001), suggesting dissimilar microbiota composition between MDD patients and controls. Analyses for each taxon using ANCOM models revealed several targets for depression, including 4 phyla (Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria), 7 families (Alcaligenaceae, Bifidobacteriaceae, Lachnospiraceae, Peptostreptococcaceae, Porphyromonadaceae, Prevotellaceae and Streptococcaceae) and 12 genera (*Adlercreutzia*, *Bifidobacterium*, *Blautia*, *Clostridium XI*, *Eggerthella*, *Holdemania*, *Megamonas*, *Parabacteroides*, *Prevotella*, *Ruminococcus*, *Streptococcus* and *Sutterella*) after adjusting for platforms and fat intake%. We presented the abundance of these identified microbiota targets in Fig. 1.

Table 2
Correlations between taxa and BDI, BAI and PSS scores.

| Taxa | BDI | | BAI | | PSS | |
|------------------------|-------|---------|-------|---------|-------|---------|
| | r | p-value | r | p-value | r | p-value |
| Phylum | | | | | | |
| Actinobacteria | 0.20 | 0.091 | 0.15 | 0.248 | 0.26 | 0.046* |
| Firmicutes | 0.16 | 0.174 | 0.11 | 0.408 | 0.13 | 0.307 |
| Bacteroidetes | -0.20 | 0.089 | -0.13 | 0.325 | -0.19 | 0.148 |
| Proteobacteria | -0.22 | 0.066 | -0.20 | 0.127 | -0.29 | 0.024* |
| Family | | | | | | |
| Prevotellaceae | -0.26 | 0.028* | -0.20 | 0.133 | -0.27 | 0.035* |
| Lachnospiraceae | 0.11 | 0.344 | 0.13 | 0.314 | 0.09 | 0.492 |
| Bifidobacteriaceae | 0.15 | 0.211 | 0.13 | 0.328 | 0.23 | 0.075 |
| Alcaligenaceae | -0.32 | 0.007** | -0.19 | 0.152 | -0.34 | 0.007** |
| Streptococcaceae | 0.18 | 0.124 | 0.09 | 0.479 | 0.15 | 0.260 |
| Porphyromonadaceae | 0.24 | 0.046* | 0.28 | 0.032* | 0.18 | 0.156 |
| Peptostreptococcaceae | 0.36 | 0.002* | 0.08 | 0.531 | 0.11 | 0.381 |
| Genus | | | | | | |
| <i>Prevotella</i> | -0.26 | 0.028* | -0.20 | 0.133 | -0.27 | 0.035* |
| <i>Blautia</i> | 0.34 | 0.004** | 0.30 | 0.020 | 0.34 | 0.007** |
| <i>Bifidobacterium</i> | 0.15 | 0.211 | 0.13 | 0.328 | 0.23 | 0.075 |
| <i>Sutterella</i> | -0.32 | 0.007** | -0.19 | 0.152 | -0.34 | 0.007** |
| <i>Ruminococcus</i> | 0.30 | 0.010* | 0.24 | 0.063 | 0.34 | 0.007** |
| <i>Streptococcus</i> | 0.19 | 0.111 | 0.10 | 0.460 | 0.15 | 0.260 |
| <i>Megamonas</i> | -0.19 | 0.116 | -0.11 | 0.423 | -0.23 | 0.079 |
| <i>Parabacteroides</i> | 0.24 | 0.046* | 0.28 | 0.031* | 0.18 | 0.158 |
| <i>Eggerthella</i> | 0.35 | 0.002** | 0.32 | 0.014* | 0.32 | 0.011* |
| <i>Adlercreutzia</i> | 0.07 | 0.583 | 0.12 | 0.361 | 0.08 | 0.536 |
| <i>Holdemania</i> | 0.13 | 0.285 | 0.22 | 0.093 | 0.38 | 0.003** |
| <i>Clostridium XI</i> | 0.26 | 0.028* | 0.15 | 0.271 | 0.07 | 0.615 |

BDI, Beck Depression Inventory; BAI, Beck Anxiety Inventory; PSS, Perceived Stress Scale.

* P-value < 0.05.

** P-value < 0.01.

3.3. Correlations of microbiota abundance and clinical characteristics

Identified microbiota targets were examined for their correlations with depression, anxiety, and stress levels. Most of the correlations showed similar trend in subgroups of the samples, thus, we presented Spearman's correlation coefficients among overall samples in Table 2, and detailed results by subgroups were displayed in Supplementary Table 2. In particular, family Peptostreptococcaceae ($r = 0.36$, p -value = 0.002) showed moderate correlations with BDI score and the trend was quite similar in patients and controls. At genus level, *Blautia* ($r = 0.34$, p -value = 0.004), *Sutterella* ($r = -0.32$, p -value = 0.007), and *Eggerthella* ($r = 0.35$, p -value = 0.002) showed moderate correlations with BDI score. In addition, genus *Eggerthella* ($r = 0.35$, p -value = 0.002), *Blautia* ($r = 0.34$, p -value = 0.004) and *Ruminococcus* ($r = 0.30$, $r = 0.01$) showed moderate correlation with PSS score. For taxa that had p -value < 0.01 and the trend was the same in patient and control groups, we presented the correlation plots in Fig. 2. Notably, *Holdemania* revealed significant correlations with BAI and PSS scores ($r = 0.65$ and $r = 0.49$, respectively) mainly in MDD patients.

3.4. Functional analysis

In the functional pathway analyses, we found 31 pathways that were enriched for MDD (q -value ranged from 0.00003 to 0.037), including compound metabolism (e.g. Amino acid, carbohydrate, starch, sucrose and galactose metabolism, etc.), biosynthesis processes (e.g. lysine, primary and secondary bile acid, phenylalanine, tyrosine and tryptophan) and degradation (e.g. bisphenol and nitrotoluene, etc.) pathways. The detailed results are displayed in Fig. 3. Comparisons of functional results with previous studies using different experimental platforms were displayed in Supplementary Table 2 (Chen et al., 2018b; Zheng et al., 2016) with either animal or human data. In brief, there were two pathways that were consistently reported for depression,

including the pentose phosphate pathway, and starch and sucrose metabolism pathway.

4. Discussion

Our study provides the first-line of results by examining gut microbiota composition between MDD patients and controls while considering the dietary information. Twenty-three targets were identified, and one of them simultaneously show moderate correlations with anxious and perceived stress level. Results of the functional pathway analyses revealed important microbiota functions for MDD, which involve with metabolisms, biosynthesis and degradation processes.

In the present study, a number of microbiota targets in phylum, family and genus levels showed different distributions between MDD patients and controls. In the literature of human clinical microbiota studies, we noted very few consistent results for depression (Kuo and Chung, 2018). Comparing with previous human microbiota studies in Chinese populations, at phylum level, Actinobacteria was consistently more abundant in patients than controls (Zheng et al., 2016; Chen et al., 2018a). However, other phyla (Proteobacteria, Bacteroidetes and Firmicutes) showed less consistent trend across studies (Jiang et al., 2015; Lin et al., 2017; Liu et al., 2016; Zheng et al., 2016). Within family level, a number of taxa showed the same abundance trend in our studies and one of previous studies, including Streptococcaceae (Zheng et al., 2016) and Bifidobacteriaceae (Chen et al., 2018a) that exhibited higher abundance in MDD patients, and Prevotellaceae (Kelly et al., 2016) that had lower abundance in patients. To our best knowledge, some targets at the family level were never reported in the literature for depression or psychiatric disorders, including Alcaligenaceae, Porphyromonadaceae and Peptostreptococcaceae. At genus level, we found some encouraging and emerging results with similar trend across studies, including *Holdemania* (Kelly et al., 2016), *Eggerthella* (Chen et al., 2018a; Kelly et al., 2016), *Streptococcus* (Lin et al., 2017), and *Sutterella* (Chen et al., 2018a; Liu et al., 2016), while many other reported genera targets showed different directions of the associations for depression, such as *Clostridium XI*, *Parabacteroides*, *Blautia*, and *Prevotella*. This various findings might be caused by diverse clinical characteristics in different samples, such as depressive severity or comorbidity conditions among patients. Other sources of the heterogeneity may also contribute to different results, including study design, ethnicity, and unmeasured confounding factors. It is important to obtain more robust findings to serve as reliable biomarkers before further exploration about their functions and involvement for depressive illness.

For associations between taxa and clinical traits, we found that many of the identified microbiota targets also correlated with either depressive severity, anxiety, or perceived stress levels, and the majority of the correlations have similar trends in patients and controls (Supplementary Table 2). One exception is genus *Holdemania*, which shows a moderate correlation with anxiety ($r = 0.22$) and perceived stress levels ($r = 0.38$). Interestingly, the associations are more prominent in patients than controls, such that the correlation coefficients are 0.65 for anxiety and 0.49 for stress in MDD patients. Because the two clinical features (anxiety and stress) are prevalent in MDD patients and important moderating factors for depression prognosis and functional outcomes (Ballenger, 2000; Burke et al., 2005; Sartorius et al., 1996), the biological functions of *Holdemania* warrant further investigation. A prospective study design is also preferable to uncover the functional relations between *Holdemania* abundance and mood related traits. There are few previous studies ever examined the associations between microbiota and clinical features, however, no common findings are reported. For instance, a negative correlation was found for genus *Faecalibacterium* and depressive severity (Jiang et al., 2015). In addition, with gender stratified analyses, Chen et al. (2018a) reported different targets to be associated with depressive severity in females (*Erysipelotrichaceae incertae sedis*, *Clostridium XIVa* and *Streptococcus*) and males (*Veillonella* and *Collinsella*). Notably, all these findings were

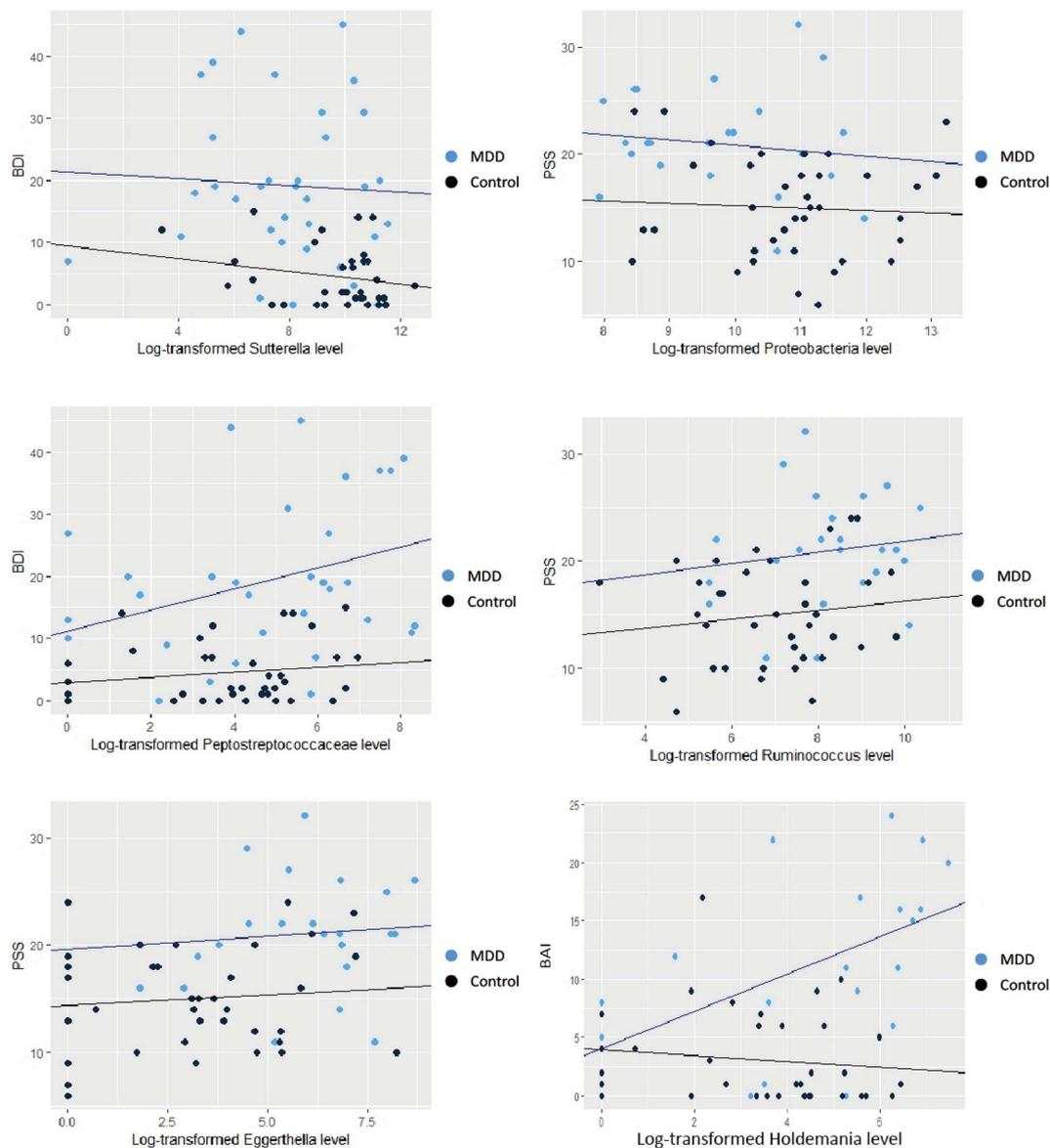


Fig. 2. The correlations of taxa and BDI, BAI and PSS scores in overall samples stratified by patients and controls.

examined only among MDD patients. In the present study, we assessed mood related traits in both MDD patients and healthy controls, and reported that similar trends can be seen also in healthy controls. Therefore, the effects of microbiota on mood might be more general phenomenon and non-specific to diagnosis.

Gut microbiota serve important functions in metabolism of nutrient and food components. We found that MDD patients consumed more carbohydrate comparing to controls, and it may not be surprised that the enriched microbiota functional pathways for depression are involved with several carbohydrate metabolism pathways. In particular, the pentose phosphate pathway, and starch and sucrose metabolism pathway (Supplementary Table 2), are common findings across different microbiota studies of depression (Chen et al., 2018b; Zheng et al., 2016). It is noted that a prospective human study showed an increased trend of depression incidence with higher sucrose intake (Gangwisch et al., 2015). Moreover, disturbances of the downstream glucose metabolism are also implicated in the etiology of depression. The risk of depression is significantly higher in patients with diabetes comparing to the general population (Nouwen et al., 2010). Interestingly, metabolism of glucose through the pentose phosphate pathway can influence a diverse of pathophysiologicals, such as conditions related to oxidative

stress, inflammation, and nitrogen species damage (Marin-Valencia et al., 2012; Perl et al., 2011), which are linked with MDD (Rawdin et al., 2013). In particular, the pentose phosphate shunt generates nicotinamide adenine dinucleotide phosphate (NADPH), which protects against oxidative and nitrosative stress. Higher activity in the key enzyme (glucose-6-phosphate dehydrogenase) of pentose phosphate pathway was found in a depression rat model (Detka et al., 2015) and in the inferior temporal cortex of Alzheimer patients (Palmer, 1999). These aforementioned observations may indicate two possibilities for the enriched carbohydrate metabolism pathways in depression, one is that the microbiota compositions are the consequences of food preference in depressive patients, the other is that microbiota play roles in more upstream etiological mechanism of depression that involve with dysfunctions of glucose metabolism. However, how exactly the biological processes underlying these mechanisms to influence depression warrant further investigations.

In our results, there are no difference between patients and controls in alpha diversity. However, the PERMANOVA analysis showed significant differences in beta diversity. The diversity of microbiota between patients and controls are not always clear in the literature. Greater value of Shannon index was found in MDD patients than

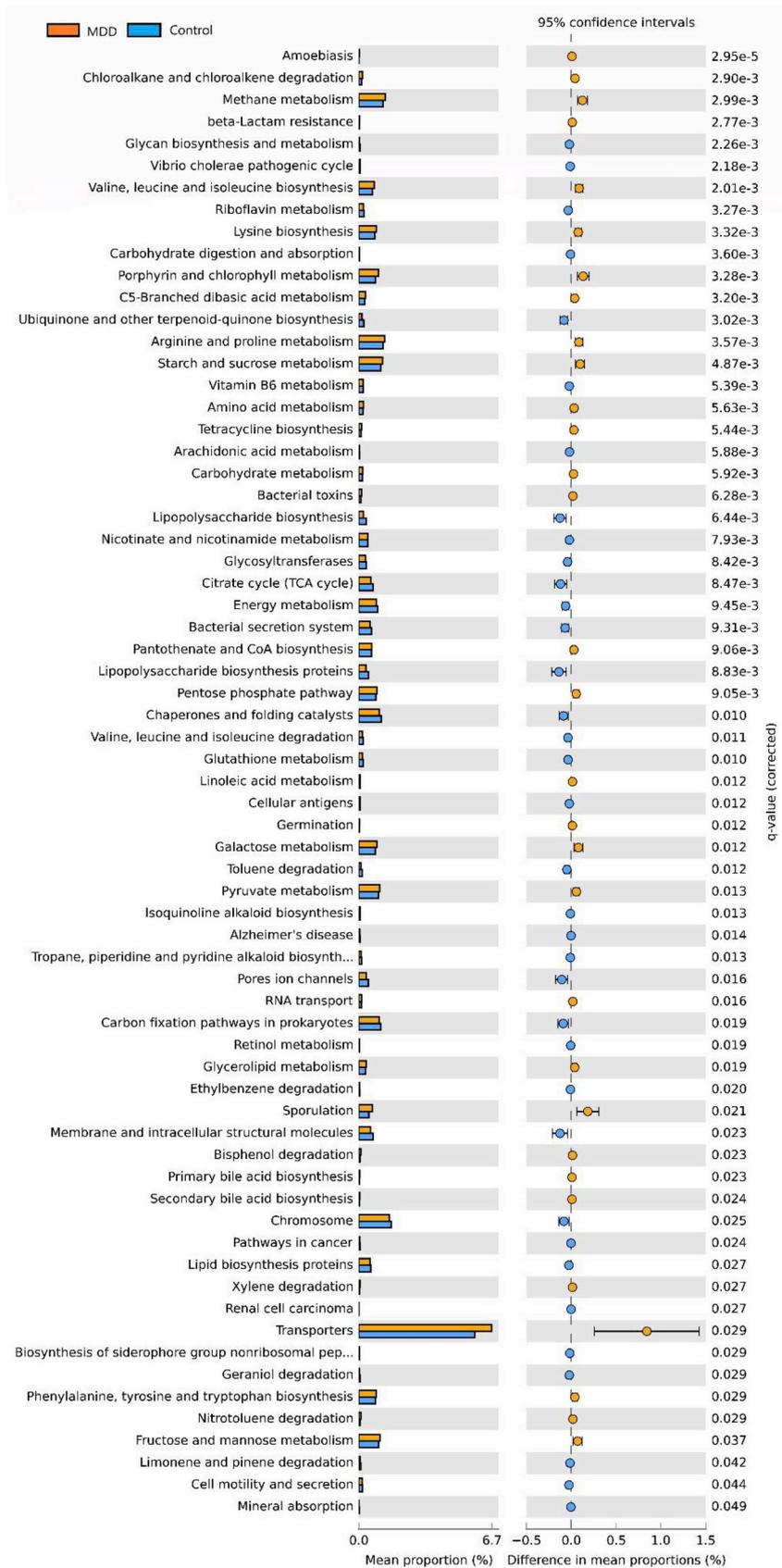


Fig. 3. PICRUST analysis for functional pathways in MDD patients and controls.

controls in one study (Jiang et al., 2015). Some studies found an opposite trend in either Shannon or chao1 indices (Kelly et al., 2016; Liu et al., 2016), while other studies reported no differences in diversity indices between cases and controls (Lin et al., 2017; Naseribafrouei et al., 2014). Similar situation was observed in beta diversity, for which some studies showed clear separation between groups (Kelly et al., 2016; Lin et al., 2017), but not in other studies (Jiang et al., 2015; Liu et al., 2016).

Although we controlled for fat intake% within the current study, there is a body of the literature evaluated the relationships between diet and specific taxa. For instance, the genus *Sutterella* was more abundant in control mice than high-fat diet feeding mice (Javurek et al., 2017). In human samples, 10-weeks intervention for resistant starch diet showed association with genus *Ruminococcus* (Walker et al., 2011). Another line of studies considered broad dietary pattern and microbiota, such that *Prevotella* is associated with plant-based dietary pattern (De Filippis et al., 2016; Mitsou et al., 2017). In the present study, the relatively moderate sample size (N = 73) may preclude us from obtaining more stable results with cluster analysis (usually hundreds of subjects) to further discuss the impacts of dietary patterns on microbiota compositions. It will be great to refine our analysis in the near future with bigger sample size to incorporate more food items or using cluster based method to capture a broader aspect of diet effects on microbiota taxa.

There are limitations in interpreting the results of the present study. Firstly, we conducted a cross-sectional study design, which hinders from making causal inference between the microbiota alterations and the risk of depression. In addition, the sample size is only moderate and we may not have enough power to identify microbiota targets with small effects. Secondly, all of the MDD patients have undergone antidepressant treatment, we cannot exclude the possibility that medication may have influenced on microbiota abundance. Although results from a previous study comparing microbiota composition between drug naïve patients and patients receiving antidepressant treatment demonstrated no significant differences due to treatment (Zheng et al., 2016), further studies are needed to more comprehensively evaluate drug effects on specific microbiota targets. Thirdly, the dietary information obtained from FFQ is subjective to recall bias. The precision of dietary intake can be benefit from having more objective dietary records in future studies.

In conclusion, we identified 23 microbiota targets to be associated with MDD. At genus level, *Holdemania*, *Eggerthella*, *Streptococcus*, and *Sutterella* are also reported in previous clinical studies for depression with more consistent trend in association direction. In addition, at genus level, 7 taxa were further correlated with depression severity, 4 were correlated with anxiety, and 6 were correlated with stress level among patients and controls. Studies with larger sample size and further integrating with metabolomics data are of interests to validate and expand the current findings for MDD and mood related traits to provide a better understanding about the link between microbiota targets and the underlying metabolic pathways involving with the etiology of depression.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jpsychires.2019.01.016>.

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Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Contributors

The authors contributed to the work substantially. Po-Hsiu Kuo and Wei-Liang Shih conceived and designed the study, critically revised the manuscript, and owned primary responsibility for the final content. Yu-Chu Ella Chung coordinated the progress of the study, conducted experiments, analyzed the data, and wrote the manuscript. Meei-Shyuan Lee provided the Taiwanese version of the food frequency questionnaire and supervised the analysis for dietary patterns. Hsiang-Chin Lori Chou performed analyses for the food frequency questionnaire. Yen-Wenn Liu provided technical supports for experimental procedures. Hsi-Chung Chen, I-Ming Chen, Mong-Liang Lu, Chun-Hsin Chen, Chi-Shin Wu, Ming-Chyi Huang, and Shih-Cheng Liao assisted for participants' referral and recruitment. Li-Chung Chuang assisted in microbiota data analysis. Yen-Hsuan Ni, and Mei-Shu Lai assisted in the design of the study.

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