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Comparison of serum microbiome composition in bipolar and major depressive disorders

Sang Jin Rhee[a,b], Hyeyoung Kim[a,b,1], Yunna Lee[a,2], Hyun Jeong Lee[c], C. Hyung Keun Park[a,b,3], Jinho Yang[d], Yoon-Keun Kim[d], Sungmin Kym[e], Yong Min Ahn[a,b,f,*]

[a] Department of Neuropsychiatry, Seoul National University Hospital, Seoul, Republic of Korea
[b] Department of Psychiatry and Behavioral Science, Seoul National University College of Medicine, Seoul, Republic of Korea
[c] Department of Psychiatry and Behavioral Science, National Cancer Center, Division of Cancer Management Policy, National Cancer Center, Goyang, Republic of Korea
[d] MD Healthcare Inc., Seoul, Republic of Korea
[e] Department of Internal Medicine, Inje University Haeundae Paik Hospital, Inje University College of Medicine, Busan, Republic of Korea
[f] Institute of Human Behavioral Medicine, Seoul National University Medical Research Center, Seoul, Republic of Korea

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ABSTRACT

Bipolar disorder and major depressive disorder are debilitating psychiatric conditions which can be difficult to differentiate; however, recent studies have suggested that microbiome composition may be a potential tool in distinguishing between these two disorders. This study aimed to compare the serum microbiome composition of patients with bipolar disorder, major depressive disorder, and healthy controls. Serum samples were collected from 42 subjects with bipolar disorder, 30 with major depressive disorder, and 36 healthy controls. Bacterial DNA was isolated from bacteria-derived extracellular vesicles in the serum and then amplified and quantified with primers specific to the V3–V4 hypervariable region of the 16S rDNA gene. Sequence reads were clustered into operational taxonomic units and classified using the SILVA database. Alpha and beta diversity, individual taxa analysis based on phylum and genus, and functional pathways were compared. There was no statistical difference between alpha or beta diversity in patients with bipolar disorder and major depressive disorder; however, the *Prevotella* 2 and *Ruminococcaceae* UCG-002 genera were significantly more prevalent in patients with major depressive disorder than in either those with bipolar disorder or in healthy controls. Functional analysis of pathways revealed that the apoptosis function differed between all three groups. In conclusion, the *Prevotella* 2 and *Ruminococcaceae* UCG-002 genera were identified as potential candidates for distinguishing bipolar disorder and major depressive disorder. Further studies with larger sample sizes, longitudinal designs, and control for other various confounders are warranted.

1. Introduction

Bipolar disorder (BD) and major depressive disorder (MDD) are prevalent and debilitating psychiatric conditions which increase socio-economic burdens (Cloutier et al., 2018; Greenberg et al., 2015) and mortality rates (Reutfors et al., 2018; Staudt Hansen et al., 2019). As clinical manifestations of the depressive phases of these disorders are similar, they are sometimes difficult to differentiate. Indeed, the mis-diagnosis rate of BD as MDD is almost 40% (Ghaemi et al., 2000), resulting in the use of antidepressant monotherapy in BD patients which increases the risk of hypomanic/manic states (Fornaro et al., 2018). The importance of making a correct distinction between these disorders cannot be overstated; therefore, considerable effort is being made to find objective biomarkers to help distinguish these disorders.

Recently, efforts to understand the role of the microbiome in human health and diseases have been extended to psychiatric disorders through the notion of the "gut-brain axis." Increasing evidence shows that gut microbiota can influence brain function via the vagus nerve, short chain fatty acids, and other immune components (Nguyen et al., 2018). Most studies concerning the influence of the microbiome on

* Corresponding author. Department of Neuropsychiatry, Seoul National University Hospital, Seoul, Republic of Korea.
E-mail addresses: hellojr1123@hanmail.net (S.J. Rhee), kimhy.md@gmail.com (H. Kim), ynlee8317@gmail.com (Y. Lee), hjlee.np@gmail.com (H.J. Lee), hkpark@gmail.com (C.H.K. Park), ykkim@mdhc.kr (Y.-K. Kim), smkimkor@paik.ac.kr (S. Kym), aym@snu.ac.kr (Y.M. Ahn).
[1] Present address: Department of Psychiatry, Inha University Hospital, Incheon, Republic of Korea.
[2] Present address: Department of Psychiatry, Kosin University Gospel Hospital, Busan, Republic of Korea.
[3] Present address: Department of Psychiatry, Asan Medical Center, Seoul, Republic of Korea.

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mood disorders have analyzed the microbiotic differences between BD or MDD patients and healthy controls (HCs), with mixed results regarding overall diversity and differences at the level of individual taxa (Chen et al., 2018; Chung et al., 2019; Coello et al., 2019; Evans et al., 2017; Jiang et al., 2015; Lin et al., 2017; Liu et al., 2016; McIntyre et al., 2019; Naseribafrouei et al., 2014; Nguyen et al., 2018; Painold et al., 2019). Rong et al. (2019) analyzed the fecal microbiome of BD and MDD patients and HCs, and found significant differences in the abundances of eight species between the BD and MDD patients; however, no significant differences were found in abundances among the genera (Rong et al., 2019).

Our current understanding of variations in the blood microbiome and their relationship to mood disorders is limited. Olde Loohuis et al. (2018) compared the whole-blood microbiomes of patients with schizophrenia, BD, and amyotrophic lateral sclerosis with those of HCs, and found that alpha diversity was significantly higher in patients with schizophrenia than in the other groups, but found no significant differences between patients with BD and HCs (Olde Loohuis et al., 2018). To our knowledge, no studies have yet compared the serum microbiome composition of patients with BD and MDD. Thus, we analyzed the differences between the serum microbiomes of patients with BD and MDD compared with those of HCs. Based on previous studies, we hypothesized that overall measures of diversity would be similar among these groups, but that individual microbial taxa would differ significantly between patients with BD and MDD.

2. Materials and methods

2.1. Study participants

In total, 72 patients (42 with BD, 30 with MDD) and 36 HCs were enrolled from Seoul National University Hospital and Inje University Haeundae Paik Hospital, respectively, from 2015 to 2018. Ages ranged from 19 to 60 years. The BD and MDD patients were selected from the out-patient psychiatric clinic of Seoul National University Hospital. Diagnoses were made according to the criteria of the Diagnostic and Statistical Manual of Mental Disorders 4th or 5th version (DSM-IV, or DSM-5), and confirmed by the Mini-International Neuropsychiatric Interview (MINI). Symptom severity was assessed using the 17-item Hamilton Depression Rating Scale (HAM-D) (Hamilton, 1960) and the 11-item Young Mania Rating Scale (YMRS) (Young et al., 1978). HCs were selected from those undergoing routine physical checkups at Inje University Haeundae Paik Hospital with no history of psychiatric disorders. Serum samples were obtained from each subject.

Patients and HCs were excluded from the study according to the following criteria: those taking antibiotics, antifungal agents, or steroids and those diagnosed with hypertension, diabetes, rheumatoid diseases, inflammatory bowel disorders, cancers, or gastrointestinal diseases. BD patients with current hypomanic, manic, or mixed episodes were also excluded, as were patients with concordant diagnoses of alcohol- or other substance-abuse disorders or eating disorders.

This study was carried out in accordance with the latest version of the Declaration of Helsinki and the study design was reviewed by the Institutional Review Boards of Seoul National University Hospital (IRB No. 1301-069-459) and Inje University Haeundae Paik Hospital (IRB No. 1297992-2015-064). Informed consent of the participants was obtained after the nature of the procedures had been fully explained.

2.2. Microbiome analysis

2.2.1. Extracellular vesicle isolation and DNA extraction from human serum samples

All samples were transferred to a serum separator tube and centrifuged at 3000 rpm and 4 °C for 15 min. The supernatant was collected and stored at −80 °C until use, at which time it was mixed with phosphate-buffered saline (PBS) and centrifuged at 10,000 × *g* and 4 °C for 10 min. Bacteria and foreign particles were thoroughly eliminated using a 0.22 μm filter. The supernatant was boiled at 100 °C for 40 min to extract DNA from extracellular vesicles (EVs); centrifuged at 13,000 rpm and 4 °C for 30 min; and then the supernatant was collected. EV DNA was isolated using a DNeasy PowerSoil Kit (QIAGEN, Germany) and quantified using QIAxpert (QIAGEN).

2.2.2. Bacterial metagenomic analysis using EV DNA from human serum samples

Libraries were prepared from the EV DNA PCR products according to the MiSeq System guide (Illumina, USA) and quantified using QIAxpert (QIAGEN). To amplify the V3–V4 hypervariable region of the 16S rDNA gene, 16S_V3_F (5′-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG CCT ACG GGN GGC WGC AG-3′) and 16S_V4_R (5′-GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GGA CTA CHV GGG TAT CTA ATC C-3′) primers were used. Each amplicon was then quantified, set at an equimolar ratio, pooled, and sequenced on a MiSeq (Illumina).

2.2.3. Microbiome bacterial composition analysis

Paired-end reads matching the adapter sequences were trimmed by Cutadapt version 1.1.6 (Martin, 2011). The resulting FASTQ files were merged using CASPER and quality filtered using the Phred (Q) score-based criteria described by Bokulich (Bokulich et al., 2013; Kwon et al., 2014). Any merged reads shorter than 350 bp and longer than 550 bp were discarded. Chimeric sequences were identified using a reference-based chimera detection step with VSEARCH against the SILVA gold database (Quast et al., 2012; Rognes et al., 2016). Next, the sequence reads were clustered into operational taxonomic units (OTUs) using VSEARCH with a de novo clustering algorithm at a 97% sequence similarity threshold. OTUs with one sequence in only one sample were excluded from further analysis. Representative sequences of the OTUs were classified using the SILVA 128 database with UCLUST (parallel_assign_taxonomy_uclust.py script in QIIME version 1.9.1) under the default parameters (Caporaso et al., 2010).

2.3. Statistical analysis

Significant differences between the BD, MDD, and HC samples were examined using the Chi-square test for categorical variables, *t*-tests or analysis of variance (ANOVA) for continuous variables, and linear regression or analysis of covariance (ANCOVA) for continuous variables when age and sex were adjusted as covariates.

Alpha diversity was calculated by the number of observed OTUs, the Chao-1 index, the inverse Simpson index, and the Shannon index. The observed OTU and Chao-1 index data were log-transformed, and Shannon index data were exponentially-transformed. Beta diversity was calculated with the Bray-Curtis dissimilarity index and both unweighted and weighted UniFrac distance metrics. Principal coordinate analysis (PCoA) based on the Bray-Curtis measure was used to visualize between-sample beta diversity relationships. Each beta diversity measure was analyzed using permutational ANOVA (PERMANOVA).

Descriptive analysis was carried out on individual phyla between the groups. Pairwise analysis of microbiome composition (ANCOM; Mandal et al., 2015) was conducted to determine significantly different genera between two groups. Genera with a mean abundance $<0.01\%$ in any group were excluded from analysis, as were genera with $>90\%$ zero values.

To predict functional pathway profiles, we carried out functional community profiling on the 16S rRNA data using Tax4Fun (Asshauer et al., 2015), which annotates metabolic cycles and pathways in the Kyoto Encyclopedia of Genes and Genomes (KEGG; Ogata et al., 1999). Statistical Analysis of Metagenomic Profiles (STAMP; Parks et al., 2014) software was used to analyze functional differences between the BD and MDD samples. The Benjamini-Hochberg procedure was used for multiple comparisons to control the false discovery rate. Functions that

Table 1
Demographic and clinical factors of the study subjects.

	BD (n = 42)	MDD (n = 30)	HC (n = 36)	Statistics	p-value†	Post-hoc[a]
Age, mean ± *SD*, years	34.2 ± 10.8	46.2 ± 9.7	43.0 ± 5.6	F = 17.48	<0.001***	MDD > BD, HC > BD
Sex						
Male, *n* (%)	15 (35.7)	5 (16.7)	9 (25.0)	χ^2 = 3.33	0.19	
BMI, mean ± *SD*, kg/m^2	24.3 ± 4.0	24.4 ± 4.3	23.9 ± 3.9	F = 0.12	0.88	
WC, mean ± *SD*, cm						
Exercise, *n* (%)	19 (45.2)	13 (43.3)		χ2 = 0.26	0.87	
Current smoker, *n* (%)	6 (14.3)	7 (23.3)		χ2 = 0.97	0.33	
Alcohol use, *n* (%)	18 (42.9)	13 (43.3)		χ2 = 0.002	0.97	
HAM-D, mean ± *SD*	6.21 ± 4.80	6.07 ± 5.43		t = 0.12	0.90	
YMRS, mean ± *SD*	3.12 ± 3.65	1.80 ± 2.71		t = 1.68	0.10	
Medication						
Antidepressant, *n* (%)	6 (14.3)	26 (86.7)		χ2 = 37.13	<0.001***	
Anticonvulsant or Lithium, *n* (%)	32 (76.2)	1 (3.3)		χ2 = 37.42	<0.001***	
Antipsychotics, *n* (%)	33 (78.6)	13 (43.3)		χ2 = 9.42	0.002**	

Significant differences between the BD, MDD, and HC samples were examined using the Chi-square test for categorical variables, and t-tests or analysis of variance (ANOVA) for continuous variables.
Abbreviations: BD = Bipolar disorder, MDD = Major depressive disorder, HC = healthy control, SD = standard deviation, BMI = body mass index, WC = waist circumference, HAM-D = Hamilton depression rating scale, YMRS = Young mania rating scale.
† p-values are significant at α = 0.05 (*), 0.01 (**), and 0.001(***).
[a] Post-hoc analysis using Tukey method.

differed significantly between BD and MDD groups were compared pairwise to HC.

All significance tests were two-sided and statistical significance was set at $\alpha = 0.05$. For the ANCOM *W* statistic, an initial threshold for rejecting the null hypothesis of differential abundance between two groups was set at 60% of comparisons; an additional analysis was conducted at a 70% threshold.

Statistical analyses were performed using R version 3.5.0.

3. Results

3.1. Demographic and clinical characteristics

A comparison of the demographic and clinical characteristics of the BD, MDD, and HC groups (Table 1) shows a significant difference in average age between the groups ($F = 17.48$, $p < 0.001$); post-hoc analysis revealed that the average ages of the MDD and HC groups were higher than that of the BD group. Symptom severity did not differ between the BD and MDD groups; however, psychiatric medication usage was significantly different between these groups.

3.2. Diversity analysis

The observed OTUs, Chao-1 index, inverse Simpson index, and Shannon index did not differ between the BD and MDD groups; however, the inverse Simpson index was higher in the BD and MDD groups than in the HCs (BD vs HC: 39.47 vs 28.09, $p < 0.001$; MDD vs HC: 44.75 vs 28.09, $p < 0.001$), and the Shannon index was higher in the MDD group than in the HCs (4.21 vs 3.84, $p < 0.001$; Table 2). Significance results did not change under ANCOVA, with adjustments made for age and sex, relative to those obtained by ANOVA (Supplementary Table 1).

No significant differences in beta diversity were determined between the BD and MDD groups by PERMANOVA. The Bray-Curtis dissimilarity and unweighted UniFrac distance differed between both the BD and MDD groups and the HCs, while the weighted UniFrac distance was different only between the BD group and HCs (Table 3). The PCoA plot based on the Bray-Curtis dissimilarity matrix is shown in Fig. 1. Following age and sex adjustment, there was still a difference between the three groups (Supplementary Table 2).

3.3. Individual taxonomic analysis

Relative abundances of the Firmicutes and Bacteroidetes phyla were higher in patients with BD and MDD than in HCs, with Firmicutes being the most abundant phylum in all three groups. The second most abundant phylum in BD patients and HCs was Proteobacteria, whereas the second most abundant phylum in MDD patients was Bacteroidetes (Fig. 2).

Statistical analysis of microbiotic community composition was performed at the genus level. When controlling for age and sex, ANCOM revealed that the *Prevotella* 2 and *Ruminococcaceae* UCG-002 genera were significantly more prevalent in patients with MDD than in BD patients and HCs. Further pairwise analysis between the patients with BD and HCs, and between the patients with MDD and the HCs revealed other differences among genera (Table 4). Results for the unadjusted and adjusted analyses with full taxa can be found in Supplementary Tables 3 and 4.

3.4. Functional analysis between BD and MDD

Functional analysis of pathways revealed that seven pathways differed significantly between the patients with BD and MDD (Fig. 3). Ascorbate and aldarate metabolism was enriched in the BD group, with all other identified functions enriched in the MDD group. Adjusting for age and sex did not change the significance of these results; thus, we compared the significance of these functions pairwise between the two patient groups and the HCs (Supplementary Figs. 1 and 2). The apoptosis function was significantly enriched in the BD group compared to the HCs (adjusted *p*-value = 0.005), and in the MDD group compared to the HCs (adjusted *p*-value < 0.001). The mineral absorption, Wnt signaling, Notch signaling, and chronic myeloid leukemia pathways were only enriched in the MDD group compared to the HCs, whereas ascorbate and aldarate metabolism was decreased in the MDD group compared to the HCs.

4. Discussion

This study was the first to compare serum microbiome composition between patients with BD and MDD. No significant differences were found between the overall diversity measures of the two groups;

Table 2
Alpha-diversity in serum microbiota.

Parameter	BD (n = 42)	MDD (n = 30)	HC (n = 36)	Statistics	p-value†	Significant pairwise comparisons[a]
Observed OTU[b], mean ± *SD*	183.57 ± 75.29	199.57 ± 74.82	200.50 ± 124.93	F = 0.47	0.62	
Chao-1 index[b], mean ± *SD*	273.58 ± 116.97	300.04 ± 111.41	252.46 ± 139.28	F = 2.45	0.09	
Inverse Simpson index, mean ± *SD*	39.47 ± 11.24	44.75 ± 9.74	28.09 ± 12.80	F = 18.87	<0.001***	MDD > HC (p < 0.001***), BD > HC (p < 0.001***)
Shannon index[c], mean ± *SD*	4.05 ± 0.40	4.21 ± 0.23	3.84 ± 0.46	F = 7.32	<0.001***	MDD > HC (p < 0.001***)

Significant differences between the BD, MDD, and HC samples were examined using analysis of variance (ANOVA).
Abbreviations: OTU = operational taxonomic unit, BD = Bipolar disorder, MDD = Major depressive disorder, HC = healthy control, SD = standard deviation.
†p-values are significant at α = 0.05 (*), 0.01 (**), and 0.001(***).
[a] Post-hoc analysis with Tukey method, p-values are significant at α = 0.05 (*), 0.01 (**), and 0.001 (***).
[b] Due to skewed distribution, log-transformation was performed before analysis.
[c] Due to skewed distribution, exponential transformation was performed before analysis.

however, there were differences between two bacterial genera and several functional pathways.

As this study was the first of its kind to focus on serum microbiome composition, we compared our results with previous studies that analyzed microbiome compositions of other sites. We found no significant difference between the serum microbiome alpha diversity of patients with BD and MDD, which is consistent with a previous study comparing gut microbiome alpha diversity (Rong et al., 2019). However, comparison between each patient group and the HCs yielded different results than those reported previously. Studies that compared gut microbiome alpha diversity between BD patients and HCs have reported either no difference (Coello et al., 2019; Painold et al., 2019), or decreased alpha diversity for certain measures (McIntyre et al., 2019; Rong et al., 2019). One study found no difference between the whole-blood microbiome alpha diversity of patients with BD and that of HCs (Olde Loohuis et al., 2018). In comparison, our study revealed no difference in richness, but we did detect increased alpha diversity in patients with BD according to both the Shannon and inverse Simpson indices. For the most part, studies comparing the gut microbiome of patients with MDD to that of HCs have reported no significant differences in alpha diversity (Chen et al., 2018; Chung et al., 2019; Naseribafrouei et al., 2014). However, one study reported that certain alpha diversity measures decreased in patients with MDD (Rong et al., 2019), while another reported that the Shannon index is higher in patients with active MDD than in HCs (Jiang et al., 2015). We found that the Shannon index, but not the inverse Simpson index, was higher in patients with MDD than in HCs.

Our study also found no significant difference in serum microbiome beta diversity between patients with BD and MDD, which is again consistent with a previous study comparing gut microbiome beta diversity (Rong et al., 2019). However, results of our comparisons between patients with each disorder and HCs differed from previous reports. Studies comparing gut microbiome beta diversity between patients with BD and HCs have either reported no difference (McIntyre et al., 2019; Painold et al., 2019), or a significant difference (Evans et al., 2017). Coello et al. (2019) reported a difference between the unweighted UniFrac distance, but not the weighted UniFrac distance. Our study yielded different results, with all beta diversity measures differing significantly between BD patients and HCs. Similar discrepancies in beta diversity have been reported in comparisons of MDD patients and HCs, with some studies reporting a difference (Chung et al., 2019; Lin et al., 2017), and one study reporting no difference (Jiang et al., 2015). Our study found that differences in beta diversity were dependent on which index was used to assess them.

Microbiome composition is known to vary between body sites (Lloyd-Price et al., 2016), with differences between multiple aspects of diversity (Yun et al., 2019). Studies that we have compared our results with have analyzed microbiota of the gut and whole blood, which may explain the differences between these and our results. Another explanation could be different age ranges and sex ratios between studies, which are factors known to affect microbiome composition (Buford et al., 2018; Chen et al., 2018). Although our study controlled for these characteristics, the different age range and sex ratio could still have influenced our results. Another possible variable could be the disease states of the patients. Depressive symptoms were relatively mild in our study, while those in the majority of the previous studies were more severe. Furthermore, when comparing MDD patients and HCs, only the Shannon index of alpha diversity, Bray-Curtis dissimilarity, and unweighted UniFrac distance for beta diversity were significantly different. These findings suggest the importance of reporting multiple measures in such studies, as results can differ depending on the diversity measure used. For instance, the Shannon index is influenced more by richness and rare species than the inverse Simpson index (Morris et al., 2014). Unlike Bray-Curtis dissimilarity, UniFrac distances are based on phylogeny, with the unweighted UniFrac distance only considering the presence and absence of taxa and the weighted UniFrac distance also considering relative abundance (Sarangi et al., 2019). When comparing MDD patients and HCs, we found significant differences in diversity when relative abundance was less weighted.

Analysis of individual phyla revealed that the serum microbiome was dominated by Firmicutes, Proteobacteria, Bacteroidetes, Actinobacteria, and Verrucomicrobia. The second most abundant phylum was Proterobacteria in BD patients and HCs, and Bacteroidetes in patients with MDD. However, phylum rankings in HCs differ between

Table 3
Beta-diversity in serum microbiota based on PERMANOVA.

Parameter	Statistics	p-value†	Post-hoc[a]
Bray-Curtis dissimilarity	F = 1.63	<0.001***	BD vs HC (p = 0.003**), MDD vs HC(p = 0.015*)
Unweighted UniFrac	F = 1.53	<0.001***	BD vs HC (p = 0.003**), MDD vs HC(p = 0.003**)
Weighted UniFrac	F = 1.80	0.015**	BD vs HC (p = 0.09*)

PERMANOVA with 1000 permutations was performed to analyze the differences of beta-diversity parameters between BD, MDD, and HC.
Abbreviations: PERMANOVA = Permutational analysis of variance, BD = Bipolar disorder, MDD = Major depressive disorder, HC = healthy control.
†p-values are significant at α = 0.05 (*), 0.01 (**), and 0.001(***).
[a] Post-hoc analysis using Bonferroni method, p-values are significant at α = 0.05 (*), 0.01 (**), and 0.001 (***).

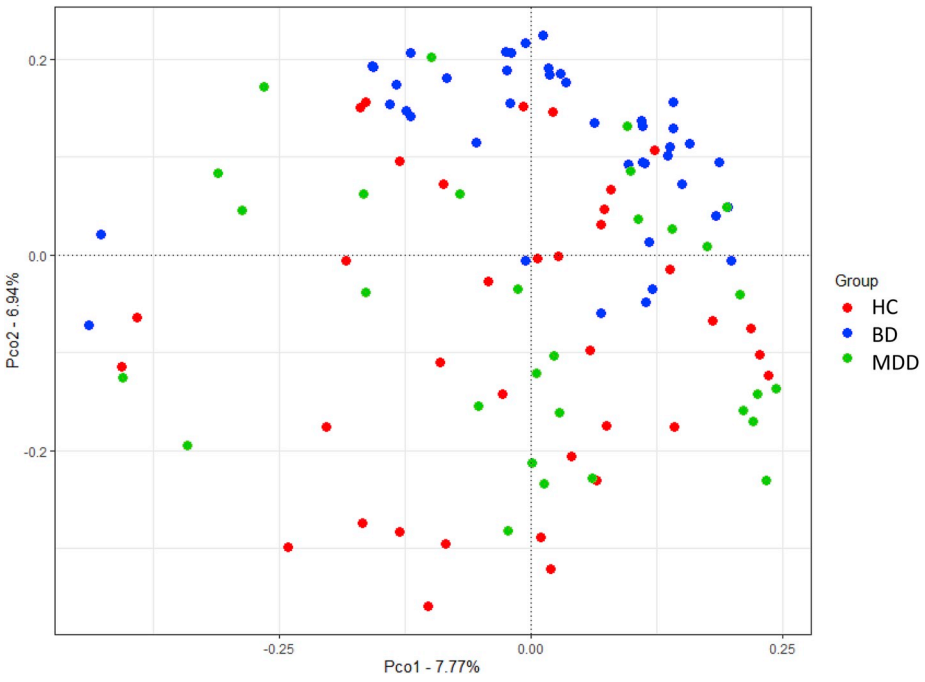

Fig. 1. Principal coordinate analysis (PCoA) plot of Bray-Curtis dissimilarity. Red, blue, and green dots represent HC, BD, and MDD groups, respectively. Plotting was based on the first two principle components PCo1 and PCo2, which accounted for 7.77% and 6.94% of the total variance. The distance between two points represents composition difference between two samples. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
Abbreviations: BD = bipolar disorder, MDD = major depressive disorder, HC = healthy control.

studies (Buford et al., 2018; Cho et al., 2019). Analysis at the genus level identified the *Prevotella* 2 and *Ruminococcaceae* UCG-002 genera as significantly more prevalent in MDD patients than in BD patients and HCs. Recently, Rong et al. (2019) reported no differences between the genera in patients with these disorders, and found that the majority of species that differed between patients with BD and MDD were in the *Prevotella* genus (Rong et al., 2019). Interestingly, that study found that *Prevotella* abundance was also higher in patients with MDD than in those with BD, which is consistent with our results.

Prevotella are anaerobic, gram-negative bacteria which are prevalent colonizers of mucosal sites (Larsen, 2017), while *Ruminococci* are anaerobic, gut-associated, gram-positive bacteria which require carbohydrates for growth (La Reau and Suen, 2018). Both of these genera are known to convert complex polysaccharides into a variety of nutrients, including short-chain fatty acids (SCFAs), which interact with intestinal epithelial cells and immune cells, and are known to influence intestinal

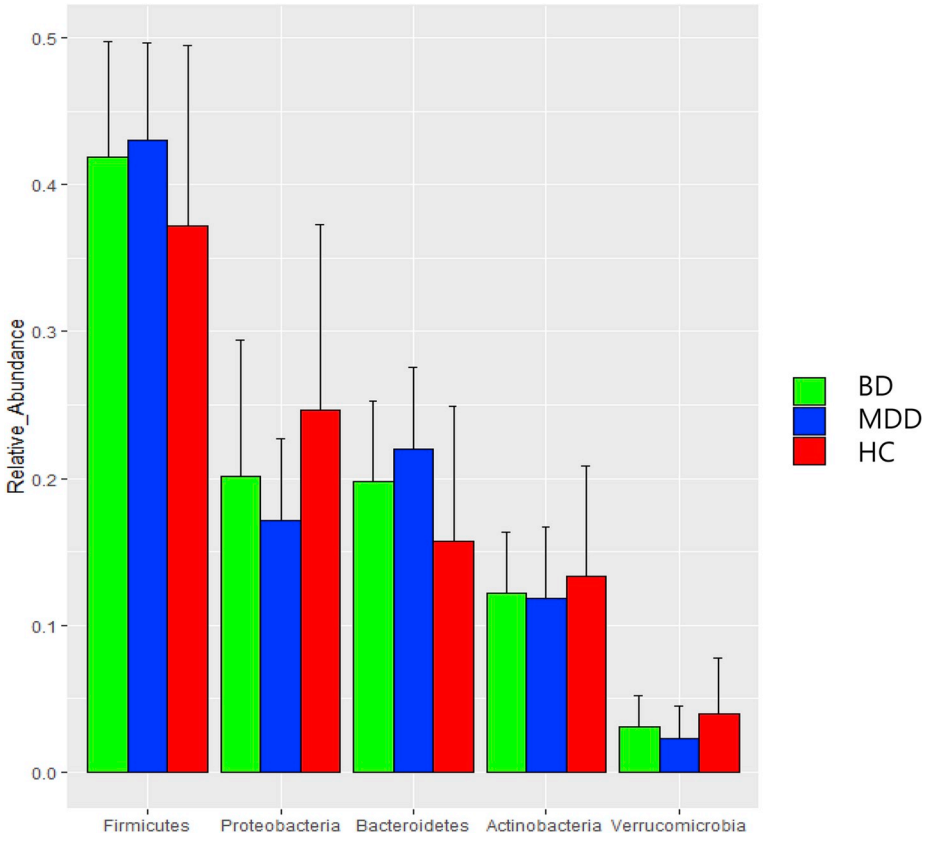

Fig. 2. The five most abundant microbiome phyla between the groups. Bar charts of relative abundances are visualized. In the BD and HC groups, the top five taxa in descending order were Firmicutes, Proteobacteria, Bacteroidetes, Actinobacteria, and Verrucomicrobia. In the MDD group, the top five taxa in descending order were Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, and Verrucomicrobia. Abbreviations: BD = bipolar disorder, MDD = major depressive disorder, HC = healthy controls.

Table 4
Individual taxa comparison of serum microbiome genus between groups.

	W statistic[a]	Relative abundance (%) mean ± *SD*		
		BD (n = 42)	MDD (n = 30)	HC (n = 36)
BPD vs MDD (n = 132)[b]				
Prevotella 2	**101**	**0.25 ± 0.54**	**1.21 ± 1.62**	0.12 ± 0.52
Ruminococcaceae UCG-002	81	**0.44 ± 0.71**	**1.12 ± 1.18**	0.49 ± 1.38
BPD vs HC (n = 132)[b]				
Faecalibacterium	**118**	**5.83 ± 3.72**	5.72 ± 2.38	**2.29 ± 2.88**
Uncultured bacterium (genus) of Chloroplast (class)	**113**	**0.21 ± 0.81**	0.39 ± 0.57	**1.80 ± 3.64**
Dialister	**113**	**1.01 ± 0.96**	1.95 ± 1.45	**0.25 ± 0.59**
Klebsiella	**112**	**1.76 ± 1.27**	1.09 ± 1.00	**0.96 ± 1.28**
Uncultured bacterium (genus) of Bacteroidales S24-7 group (family)	**102**	**3.92 ± 2.57**	4.05 ± 2.54	**2.11 ± 4.06**
Escherichia-Shigella	**97**	**3.26 ± 1.94**	3.06 ± 2.16	**1.72 ± 2.06**
Ruminococcus 2	**95**	**1.01 ± 1.16**	0.95 ± 1.21	**0.44 ± 1.13**
Uncultured (genus) of Corynebacteriaceae (family)	**94**	**1.39 ± 1.89**	1.44 ± 1.36	**0.28 ± 0.58**
Alistipes	82	**1.31 ± 1.58**	1.62 ± 1.63	**0.45 ± 1.04**
Prevotella 9	80	**3.01 ± 2.31**	4.45 ± 2.92	**1.45 ± 2.02**
MDD vs HC(n = 132)[b]				
Dialister	**123**	1.01 ± 0.96	**1.95 ± 1.45**	**0.25 ± 0.59**
Prevotella 2	**115**	0.25 ± 0.54	**1.21 ± 1.62**	**0.12 ± 0.52**
Faecalibacterium	**113**	5.83 ± 3.72	**5.72 ± 2.38**	**2.29 ± 2.88**
Pseudomonas	**111**	0.88 ± 0.91	**0.78 ± 1.40**	**3.76 ± 4.46**
Prevotella 9	**110**	3.01 ± 2.31	**4.45 ± 2.92**	**1.45 ± 2.02**
Alistipes	**108**	1.31 ± 1.58	**1.62 ± 1.63**	**0.45 ± 1.04**
Uncultured bacterium (genus) of Bacteroidales S24-7 group (family)	**107**	3.92 ± 2.57	**4.05 ± 2.54**	**2.11 ± 4.06**
Uncultured (genus) of Corynebacteriaceae (family)	**102**	1.39 ± 1.89	**1.44 ± 1.36**	**0.28 ± 0.58**
Ruminococcaceae UCG-002	**101**	0.44 ± 0.71	**1.12 ± 1.18**	**0.49 ± 1.38**
Tsukamurella	**94**	0.78 ± 1.07	**1.02 ± 1.45**	**0.31 ± 1.06**
Acidovorax	85	0.06 ± 0.15	**0.02 ± 0.08**	**0.21 ± 0.39**
Escherichia-Shigella	81	3.26 ± 1.94	**3.06 ± 2.16**	**1.72 ± 2.06**

Abbreviations: BD = Bipolar disorder, MDD = Major depressive disorder, HC = healthy control, SD = standard deviation.
Boldface are significant by the cutoff of 70%.
[a] Based on the cutoff of 60% by ANCOM(analysis of composition of microbiomes) adjusted by age and sex.
[b] Only included genus taxa with mean abundance over of 0.01% in every group, and excluded genus taxa with more than 90% of zero values.

mucosal immunity and barrier function (Dalile et al., 2019). Peripherally, SCFAs regulate interleukin circulation which influences systemic inflammation, whereas centrally they affect microglia function and thus influences neuroinflammation (Dalile et al., 2019). Consequently, these microbial genera can induce inflammatory disorders via these mechanisms. Indeed, increased *Prevotella* abundance has been observed in various inflammatory disorders, and some evidence shows that *Prevotella* strains stimulate inflammation stimulated by T helper type 17 (Th17; Larsen, 2017). Moreover, it has been demonstrated that *Prevotella* DNA is more abundant in the blood of rheumatoid arthritis patients (Martinez-Martinez et al., 2009), suggesting that this bacteria is involved in systemic dissemination (Larsen, 2017). Although there is less research regarding *Ruminococci*, some evidence suggests that this genus is associated with inflammatory bowel disease (Kang et al., 2010). The specific inflammatory pathways associated with these strains, such as Th17-mediated inflammation, could potentially help distinguish between BD and MDD. Notably, the apoptosis function was more enriched in MDD and BD patients when compared to HCs. This is consistent with the pathophysiology of inflammation and immune dysregulation in mood disorders. In particular, the apoptosis function was mostly enriched in the MDD group compared to the BD group, suggesting that the degree of apoptosis could be more significant in MDD. Although our results may have been influenced by the severity of depressive symptoms in study participants, the HAM-D score was not significantly different between the two groups. Further investigation into the associations between specific *Prevotella* and *Ruminococcus* strains and inflammation in mood disorders is needed to clarify these mechanisms.

The link between the gut microbiome and the circulating microbiome has not yet been fully elucidated; therefore, the sources of serum

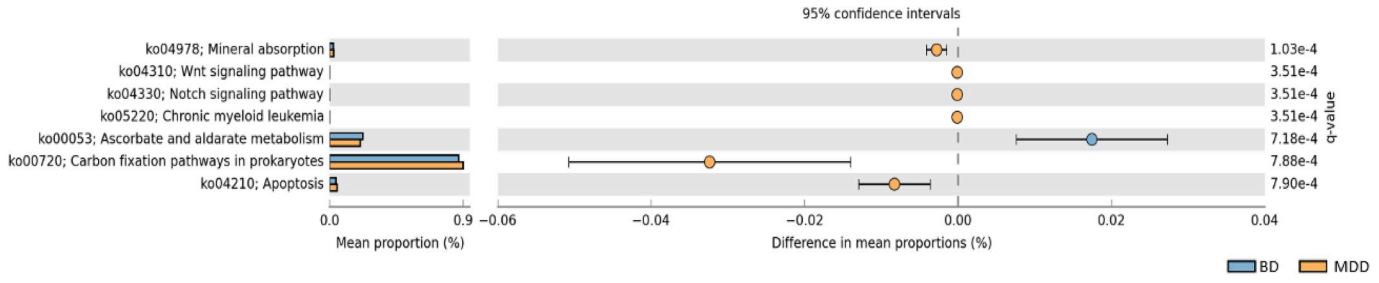

Fig. 3. Significantly different functional pathways between BD and MDD are visualized based on the Kyoto Encyclopedia of Genes and Genomes (KEGG). The mean proportion, difference in mean proportions, and 95% confidence intervals are plotted. The ascorbate and aldarate metabolism function was enriched in BD, and the rest were enriched in MDD.
Abbreviations: BD = bipolar disorder, MDD = major depressive disorder.

bacteria remain unclear. One hypothesis is that bacteria in the bowel can translocate into the blood stream. It is well known that BD and MDD are associated with inflammation and immune dysregulation (Ciobanu et al., 2018; Mao et al., 2018; Wang et al., 2018). These states can originate from gastrointestinal dysbiosis via alterations in the intestinal-vascular interface, potentially leading to the translocation of the gut microbiome into the bloodstream (Dickerson et al., 2017). Interestingly, the risk of developing BD and MDD is known to increase following irritable bowel syndrome diagnosis (Lee et al., 2015). However, circulating bacteria could also originate from areas other than the gut (Koren et al., 2011). Furthermore, it remains unclear whether circulating microbial DNA represent intact microbiomes, or if they are DNA fragments that have originated from other areas of the body.

An important finding from our study is that the ascorbate and aldarate metabolism function was significantly decreased in the MDD group compared to the BD group and HCs. Ascorbate (vitamin C) is a modulator of several neurotransmitters; for instance, the conversion of dopamine to norepinephrine requires ascorbate as a cofactor (Moretti et al., 2017). Although evidence is inconclusive, some clinical studies have shown ascorbate to be effective for treating MDD (Moretti et al., 2017). A recent report showed that levels of the urinary metabolite of ascorbic acid were reduced in an adrenocorticotrophic hormone-induced rat model of depression, and that gut microbial composition was also changed (Song et al., 2019). Ascorbate also acts as an antioxidant and an anti-inflammatory agent, which are proposed mechanisms for its therapeutic effects in BD; however, the clinical and pathophysiological association of ascorbate with BD is inconsistent (Moretti et al., 2017). The decrease in ascorbate metabolism associated with MDD could be a diagnostic feature that distinguishes patients with this disorder from not only HCs, but also from patients with BD. Thus, further investigation should be conducted regarding metabolism and microbiome composition.

This study has several limitations. First, the sample size was small and the analysis was drawn solely from a Korean population, limiting the representativeness and generalization of the results. Future studies will require a larger sample size and subjects of multiple ethnicities. Second, our study was a cross-sectional analysis; therefore causative conclusions could not be determined. Another difficulty in cross-sectional studies is that a given MDD diagnosis always has the potential to be undiscovered BD, with hypomanic/manic episodes either not yet manifested or unreported. Thus, longitudinal studies involving repetitive measures of microbiome composition are required in the future (Jiang et al., 2019). Third, we did not control for medication usage. A previous study of BD reported that the microbiomes of females treated with antipsychotics display lower species diversity than those of females not treated with antipsychotics (Flowers et al., 2017). Antidepressants are also known to have antimicrobial effects (Macedo et al., 2017). Thus, comparing drug-naïve or drug-free patients could provide greater insight. Finally, other possible confounding factors such as sampling time, diet, and physical activity were not controlled for (Liang and FitzGerald, 2017; Singh et al., 2017).

Despite these limitations, our study is the first to compare the serum microbiomes of patients with BD and MDD to those of HCs. Furthermore, the majority of our analyses controlled for age and sex, unlike most previous studies. We found that the serum microbiome may be potentially useful in differentiating between mood disorders, and we identified possible pathway functions that differ between these disorders.

In conclusion, this study demonstrated no difference in overall serum microbiome diversity between patients with BD and MDD, although differences were found at the genus level. Further studies with longitudinal designs are required to assess microbiome profiles, particularly in patients initially diagnosed with MDD who later develop hypomanic/manic symptoms. Moreover, studies with a larger sample size and multi-ethnic subjects are needed to control for other covariates which can influence serum microbiome profiles.

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CRediT authorship contribution statement

Sang Jin Rhee: Conceptualization, Formal analysis, Writing - original draft. **Hyeyoung Kim:** Conceptualization, Methodology, Writing - review & editing. **Yunna Lee:** Conceptualization, Methodology, Writing - review & editing. **Hyun Jeong Lee:** Conceptualization, Methodology, Writing - review & editing. **C. Hyung Keun Park:** Conceptualization, Methodology, Writing - review & editing. **Jinho Yang:** Methodology, Formal analysis, Writing - original draft. **Yoon-Keun Kim:** Methodology, Formal analysis, Writing - review & editing. **Sungmin Kym:** Resources, Writing - review & editing. **Yong Min Ahn:** Conceptualization, Resources, Funding acquisition, Supervision.

Declaration of competing interest

Yong Min Ahn receives research support from or serves as a speaker for Janssen Korea Ltd., Lundbeck Korea Co., Ltd, and Korea Otsuka Pharmaceutical. The other authors have no conflict of interest to declare.

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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.2020.01.004.

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