Gut Microbiota Changes in Patients with Bipolar Depression

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This study aims to characterize the gut microbiota in depressed patients with bipolar disorder (BD) compared with healthy controls (HCs), to examine the effects of quetiapine treatment on the microbiota, and to explore the potential of microbiota as a biomarker for BD diagnosis and treatment outcome. Analysis of 16S-ribosomal RNA gene sequences reveals that gut microbial composition and diversity are significantly different between BD patients and HCs. Phylum Bacteroidetes and Firmicutes are the predominant bacterial communities in BD patients and HCs, respectively. Lower levels of butyrate-producing bacteria are observed in untreated patients. Microbial composition changes following quetiapine treatment in BD patients. Notably, 30 microbial markers are identified on a random forest model and achieve an area under the curve (AUC) of 0.81 between untreated patients and HCs. Ten microbial markers are identified with the AUC of 0.93 between responder and nonresponder patients. This study characterizes the gut microbiota in BD and is the first to evaluate microbial changes following quetiapine monotherapy. Gut microbiota-based biomarkers may be helpful in BD diagnosis and predicting treatment outcome, which need further validations.

1. Introduction

Bipolar disorder (BD), a chronic and recurrent disease, has a worldwide prevalence of around 0.8%.[1,2] BD is associated with severe impairment in cognitive and social functions,[2] and increased risk of disability and suicide, especially in young individuals.[3] To date, the pathogenesis of BD has not been fully elucidated. Interactions between genetic and environmental factors may play a role,[4] along with biological alterations such as immune activation, metabolic disturbance, oxidative stress, and circadian rhythm abnormality.[5]

In recent years, the role of the brain–gut–microbiota axis in maintaining physical and mental well-being has attracted accumulated attention. As a bidirectional modulation system, this axis builds a
Hitherto only a few studies have preliminarily explored the gut microbiota in BD patients. Compared with healthy subjects, decreased abundance of Faecalibacterium in BD patients was observed, and shown to be associated with self-reported symptoms and disease severity.[12] Another study also reported decreased Faecalibacterium and Ruminococcaceae in BD patients.[13] In this study, bacterial clades associated with inflammatory status, metabolic profiles, oxidative stress, and depressive symptoms have also been identified.[13] BD patients treated with atypical antipsychotics showed altered composition of microbiota communities, especially in females.[14] In patients with newly diagnosed BD, an association between genus Flavonifractor and BD was reported, but could be confounded by factors such as smoking and female sex.[15] Interestingly, gut microbiota might participate in the pathogenesis of BD via epigenetic modifications of clock molecules (e.g., ARNTL).[16] In addition to patients with current BD, gut microbiota in individuals at high risk for BD also has been investigated.

No significant difference was found in gut bacterial constituents between unaffected first-degree relatives and healthy individuals.[15] However, in a monozygotic twin study, twins at high risk exhibited a similar pattern of gut microbial diversity to those affected by mood disorders.[17] Taken together, these studies provided initiatory evidence that the gut microbiota may be involved in the development of BD. Nonetheless, the mood state in BD patients enrolled in these studies was not clearly classified, and the influence of psychotropic medications on the gut microbiota is not strictly controlled. Moreover, whether the gut microbial markers are helpful in disease diagnosis was not evaluated either.

The aims of this study are to characterize the gut microbiota in depressed BD patients, before and after quetiapine administration, and to study microbiota associations with clinical factors and depressive severity. Furthermore, the potential of using gut microbiota as noninvasive tool for diagnosing BD and predicting treatment outcome was also explored.

2. Results
2.1. Clinical Characteristics of the Recruited Subjects

A total of 72 BD patients and 45 healthy controls (HCs) were recruited in this study, and 20 of them with 17-item Hamilton Depression Rating Scale (HDRS-17) score of $<14$ or with missed data were excluded. Finally, 52 BD patients and 45 HCs were included for further analysis. There were no significant differences in sex and BMI between the two groups. However, HCs tended to be older ($P < 0.05$). The detailed characteristics of all individuals are shown in Table 1 and Table S1 (Supporting Information). Permutational multivariate analysis of variance (PERMANOVA) showed that age was weakly associated with microbial composition only in two distances

| Table 1. Demographic and clinical details of recruited subjects (BD, bipolar disorder; H, healthy controls; BMI, body mass index; MADRS, Montgomery-Åsberg Depression Rating Scale; HDRS-17, 17-item Hamilton Depression Rating Scale; YMRS, Young Mania Rating Scale; NOS, not otherwise specified; SD, standard deviation). |
|---------------------------------|-------------------|-----------------|---------------|
| Demographic and clinical indexes | BD                | H               | P             |
| Sex                             |                   |                 |               |
| Female                          | 25 (48.08)        | 22 (48.89)      | 0.55          |
| Male                            | 27 (51.92)        | 23 (51.11)      |               |
| Age (year, mean ± SD)           | 24.15 ± 9.50      | 36.29 ± 12.22   | <0.001        |
| BMI (kg m$^{-2}$, mean ± SD)    | 21.58 ± 3.60      | 22.37 ± 2.91    | 0.77          |
| MADRS score (mean ± SD)         | 28.15 ± 8.85      | –               | –             |
| HDRS-17 score (mean ± SD)       | 30.15 ± 8.31      | –               | –             |
| YMRS score (mean ± SD)          | 1.87 ± 1.43       | –               | –             |
| Onset age (year, mean ± SD)     | 19.64 ± 7.91      | –               | –             |
| Duration of illness (year, mean ± SD) | 4.86 ± 4.69 | –               | –             |
| Bipolar diagnosis               |                   |                 |               |
| I                               | 12 (23.08)        | –               | –             |
| II                              | 38 (73.08)        | –               |               |
| NOS                             | 2 (3.85)          | –               |               |
| Family history                  |                   |                 |               |
| Yes                             | 14 (26.92)        | –               | –             |
| No                              | 38 (73.08)        | –               |               |
(unweighted unifrac distance and spearman coefficient distance with p values of 0.04 and 0.002, respectively). However, the effect of BD (cohort) was much more significant than age (Table S2, Supporting Information). Among the 52 BD patients, fecal samples were re-examined in 20 of them after quetiapine treatment. The HDRS-17 and Montgomery-Åsberg Depression Rating Scale (MADRS) scores were significantly decreased following quetiapine treatment in BD patients \( (P < 0.05) \), but no difference was observed in Young Mania Rating Scale (YMRS) scores. Demographic and clinical characteristics of the 20 treated BD patients are presented in detail in Table S3 (Supporting Information).

2.2. Sequencing Characteristics

A total of 140 samples from all the recruited subjects were sequenced on an Illumina MiSeq sequencer. For downstream analysis, 8,222,581 qualified reads from 11,705,664 raw reads were filtered. In each sample, 20,000 reads were randomly selected. Thus, 2,788,347 reads were selected from all samples (samples with more than 10,000 reads were kept). Finally, 718 qualified Operational Taxonomy Units (OTUs) were clustered for downstream analysis. Due to their low frequencies in samples, 139 OTUs were discarded (e.g., OTU contained by no more than 2 samples or not involving more than 2 reads). A total of 96.64% of all qualified reads (not randomly selected reads) were clustered into qualified OTUs generated with randomly selected qualified reads; thus, these 718 OTUs generated by randomly selected reads could cover almost all sequences. The sequencing results from 52 untreated patients, 20 treated patients, and 45 HCs were selected for the following analysis. For details, please refer to Table S4 in the Supporting Information.

2.3. Gut Microbial Diversity Changes in BD Patients Compared with HCs

Different diversity indexes (Shannon, Simpson, inverse Simpson [invSimpson], Obs, Chao 1 and Incidence-based Coverage Estimators [ICE]) were used to assess gut microbiota diversity. In this study, gut microbial diversity, as estimated by Obs, Chao 1, and ICE index, was greater in HCs compared with untreated BD patients \( (P = 0.00048, 0.0015, \text{ and } 0.00055, \text{ respectively}) \). However, no diversity changes were associated with quetiapine treatment (Figure 1A,B).

2.4. Composition at the Whole Microbiota Level

Principal coordinate analysis (PCoA) was performed with six different distances at the OTU level, focusing on the phylogenetic comparison of microbial communities. Significant differences were observed in the first five principal coordinates (PCs) between BD patients and HCs (PC1, PC2, PC3, PC4, PC5, Table S5 and Figure S1, Supporting Information; \( P < 0.05 \)).

2.5. Changes of Gut Microbiota Taxonomic Composition in BD Patients

96.7% and 88.14% of all reads were assigned into families and genera, respectively (Figure S2A, Supporting Information). At the phylum level, Bacteroidetes, Firmicutes, Proteobacteria, and Actinobacteria were the most abundant entities in the gut microbiota (Figure S2B, Supporting Information). In addition, the Bacteroides, Prevotella, Faecalibacterium, Roseburia, and Lachnospiraceae incertae sedis dominated the gut microbiota at
the genus level (Figure S2C, Supporting Information). There was higher abundance of the Firmicutes phylum in HCs, while the Bacteroidetes group was enriched in untreated BD patients. Parabacteroides, Bacteroides, Weissella, and Halomonas abundance rates were higher in untreated BD patients compared with HCs, while the Roseburia, Faecalibacterium, Ruminococcus, Gemmiger, Parasutterella, and Coprococcus genera were more abundant in HCs (P < 0.05, linear discriminant analysis [LDA] score > 2; Figure 2A,B). Further, we used permutational ANOVA test to validate the results from LDA effect size (Lefse) analysis and found that Bacteroidetes phylum, Parabacteroides, Bacteroides, and Halomonas genera were greatly enriched in patients, while Firmicutes phylum, Roseburia, Faecalibacterium, and Coprococcus genera were consistently higher in HCs. In addition, higher abundance of the Proteobacteria phylum was found following quetiapine administration in BD patients. At the genus level, Klebsiella, Lactobacillus, Anaeroglobus, Collinsella, Paraprevotella, Solobacterium, and Veillonella were enriched in treated BD patients, while Alistipes abundance was higher in untreated BD patients (P < 0.05, LDA score > 2; Figure 2C,D). Permutational ANOVA test showed higher Klebsiella and Veillonella in treated BD patients and no difference in phylum level between untreated and treated patients.

2.6. Differences of Gut Microbiota between BD-I and BD-II Subgroups Prior to Treatment

Among the 52 BD patients, there were 12 BD-I patients, 38 BD-II patients, and 2 patients classified as other types. Due to the small number of patients with other types, PCoA was performed only between BD-I and BD-II patients. PCoA analysis indicated that there existed no difference on the phylogenetic comparison

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**Figure 2.** Lefse and LDA analyses revealed differences in taxonomic composition of untreated BD patients compared to healthy controls and changes in the taxonomic composition of the gut microbiota in BD patients before and after treatment with quetiapine. A) LDA scores showing significant bacterial differences between untreated BD patients (red) and healthy controls (green). B) A cladogram of different taxonomic compositions in untreated BD patients (red) and healthy controls (green). C) LDA scores showing significant bacterial differences between untreated patients (red) and treated BD patients (green). D) A cladogram of different taxonomic compositions in untreated patients (red) and treated BD patients (green).
Figure 3. Differences in gene function in untreated BD patients compared with healthy controls, and in the BD cohort before and after treatment with quetiapine, using PICRUSt. A) Bar plots of KEGG modules are significantly different between BD patients and healthy controls. B) Bar plots of KEGG modules are significantly different in BD cohort before and after treatment with quetiapine. PICRUSt: Phylogenetic Investigation of Communities by Reconstruction of Unobserved States. KEGG: Kyoto Encyclopedia of Genes and Genomes.

2.7. Function Changes Associated with Quetiapine Treatment

Of all OTUs, 91.94% were aligned into the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) built-in reference database. Thus, a total of 5329 taxonomy and Kyoto Encyclopedia of Genes and Genomes (KEGG) orthodoxies were parsed and mapped into 118 KEGG modules. A total of 31 modules were significantly different between HCs and untreated BD patients ($P < 0.05$, LDA score $> 2$; Figure S3A, Supporting Information). Following ANOVA testing, genus Ruminococcus was also enriched in BD-II patients.

2.8. Tree-Based Classification Models

Random forest classification models were constructed with Leave-one-out (LOO) cross validation, using 30 genera in 52 patients and 45 controls ($P < 0.01$, Table S6, Supporting Information). The ratio of randomly generated decision trees fitting the input sample’s label “BD” was termed probability of BD (POBD). The POBD value was significantly increased in the BD samples versus healthy samples ($P = 1.2 \times 10^{-7}$, Figure 4A).

We also used classification models to predict the effects of quetiapine treatment in BD patients. According to HDRS-17 score reduction from baseline of at least 50% after a 4-week quetiapine treatment, BD patients were divided into two subgroups, including responders and nonresponders. Because the HDRS-17 scores for three patients were missed, 17 treated BD samples were finally included (Table S7, Supporting Information). A total of 10 genera ($P < 0.1$, Table S8, Supporting Information) in 17 samples (12 responders and 5 nonresponders) were used to construct random forest models with LOO cross validation. A significant difference was observed in responder and nonresponder BD patients ($P < 0.01$); the ROC showed an AUC of 0.93 (95% CI: 0.816–1.000; Figure 4C,D).

2.9. Associations of Gut Microbiota with Clinical Parameters

We also assessed the associations of clinical parameters with the gut microbiota. Regardless of quetiapine treatment, we found that the clinical parameters and severity of depression were closely associated with the abundance rates of bacterial genera in BD patients (Figure 5). BMI was positively with Roseburia abundance but negatively correlated with Clostridium IV, Dorea, Holdemania, Veillonella, Phascolarctobacterium,
Eggerthella, Oscillibacter, Bilophila, Flavonifractor, Solobacterium, and Corynebacterium amounts (P < 0.05). The duration of illness was positively correlated with Allisonella abundance, while negatively correlated with Escherichia/Shigella, Flavonifractor, Staphylococcus abundance (P < 0.05). In addition, MADRS scores were negatively correlated with the levels of Acetanaerobacterium, Stenotrophomonas, Anaerotruncus and Raoultella, but positively correlated with Acinetobacter and Cronobacter (P < 0.05).

3. Discussion

This study characterized the gut microbiota in depressed BD patients compared with HCs, and before and afterquetiapine treatment. Moreover, associations of gut microbiota with clinical parameters and the severity of depression were investigated. The results demonstrated that the gut microbiota’s taxonomic composition and diversity were significantly
different in BD patients. Gut microbiota compositions in untreated BD patients and HCs were dominantly characterized by Bacteroidetes and Firmicutes, respectively. Moreover, we found that several butyrate-producing genera were abundant in HCs compared to untreated BD patients. This study also revealed that the amounts of specific genera were correlated with clinical parameters and depressive severity. Notably, our results indicated that BD patients and HCs could potentially be distinguished by the gut microbiota, and treatment outcome might also be predicted by microbial markers.

As shown in the results, greater diversity of gut microbiota was found in HCs compared with BD patients; however, quetiapine treatment did not change the diversity in our samples. Gut microbiota diversity could be affected by various factors, such as diet, health status, and age, the richness in bacterial diversity is possibly protective from metabolic and autoimmune diseases. Although some studies reported no significant difference in gut microbiota diversity between major depressive disorder (MDD) patients and healthy individuals, while others found it was greater in MDD patients. A previous study has assessed the interaction between atypical antipsychotic (AAP) and gut microbiota in BD patients, and showed gender-specific reduction of species richness in AAP-treated females, which may be associated with metabolic factors.

Our results demonstrated that the microbial composition in depressed BD patients was clearly different from HCs. Phylum Firmicutes were more abundant in healthy individuals, while greater amounts of Bacteroidetes were found in untreated depressed BD patients, in accordance with a previous study in MDD patients. Of note, compared to HCs, decreased abundance of various butyrate-producing bacteria was found in untreated BD patients, including genera Roseburia, Faecalibacterium, and Coprococcus. These bacteria can produce short-chain fatty acids, such as butyrate, acetic acid, and valeric acid. Butyrate, one of the main products of colonized microbiota, serves as a major energy source for colonocytes, and plays an important role in immune accommodation, gut barrier regulation, gut metabolism, and energy modulation. Butyrate has been reported to be beneficial for many systemic disorders, such as hemoglobinopathy, genetic metabolic diseases, hypercholesterolemia, insulin resistance, and ischemic stroke. Furthermore, butyrate in the central nervous system can affect the function of hippocampus and promote the expression of...
BDNF, which has been shown to have antidepressant-like effects in animal models.\textsuperscript{[35]} Therefore, inadequate butyrate-producing bacteria in BD patients may contribute to the disease pathology. Following quetiapine treatment, gut microbial composition was remarkably changed. Klebsiella and Veillonella genera were abundant in treated patients. Previous studies observed higher levels of Klebsiella and Veillonella in MDD\textsuperscript{[26]} and BD\textsuperscript{[27]} patients, respectively. These findings provide primary evidence that the gut ecosystem in BD patients is different from health individuals, even if the depressive symptoms are alleviated. Some possible explanations for the alterations of gut microbiota in treated BD patients need to be mentioned. On the one hand, patients with spontaneous remission and drug-assisted remission may have different gut microbial communities. On the other hand, quetiapine treatment increases the risk of metabolic disturbance in susceptible patients, which may be associated with specific bacteria in the gut.

We further investigated the association between relevant clinical parameters and depressive severity with gut microbiota. Among BD patients, BMI was positively associated with Roseburia abundance, but negatively correlated with Clostridium IV, Dorea, Holdemania, Veillonella, Phascolarctobacterium, Eggerthella, Oscillibacter, Bilophila, Flavonifractor, Solobacterium, and Corinebacterium. In patients with anorexia nervosa, Clostridium abundance was shown to be negatively correlated with psychopathological scores.\textsuperscript{[28]} An animal study showed that increased abundance in Clostridium was related to antidepressant-like effects in susceptible mice.\textsuperscript{[29]} Furthermore, Clostridium IV abundance was increased after weight loss.\textsuperscript{[30]} These findings indicated that Clostridium IV might be involved in the development of BD through metabolic pathways. In addition, Phascolarctobacterium genus was found to be correlated with positive mood in healthy adults.\textsuperscript{[31]} Holdemania genus is associated with glucose metabolism and metabolic syndrome.\textsuperscript{[32]} which is highly prevalent in BD patients.\textsuperscript{[33]} Therefore, gut microbiota may participate in the metabolic perturbations in BD individuals.

Previous studies have reported a negative association between Faecalibacterium abundance and disease severity in MDD and BD patients.\textsuperscript{[12,20]} In our study, MADRS scores were negatively correlated with Acetanaerobacterium, Stenotrophomonas, Anaerotruncus, and Raoultella levels, and positively correlated with Acinetobacter and Cronobacter levels. Acetanaerobacterium and Anaerotruncus all belong to Ruminococcaceae family, which was decreased in BD patients compared with HCs.\textsuperscript{[13]} We also found higher abundance of Ruminococcaceae family in HCs. Ruminococcaceae family was reported to be correlated with energy metabolism pathways, including gluconeogenesis, glycogenolysis, and the pentose phosphate pathways.\textsuperscript{[34]} Therefore, lower Ruminococcaceae in BD patients was possibly related to abnormal glucose metabolism. Genera Stenotrophomonas and Raoultella were both members of Gammaproteobacteria class, and treated patients were richer in Gammaproteobacteria compared with untreated patients in our study. Hence, the bacteria clades associated with depressive severity may link to energy metabolism and pharmacotherapy.

Notably, this study is the first to show that BD patients and HCs can be possibly distinguished by gut microbiota. Our study has achieved satisfied classification efficacy for distinguishing BD patients from HCs based on random forest classification models. Previous studies had shown that gut microbiota could be regarded as potential noninvasive biomarkers for diagnosing nonpsychiatric diseases, such as hepatocellular carcinoma,\textsuperscript{[35]} type 2 diabetes,\textsuperscript{[36]} and colorectal cancer.\textsuperscript{[37]} Our study used optimal 30 OTUs markers based on 16S rRNA sequences, and achieved a high accuracy (AUC = 0.81) between BD patients and HCs, indicating a powerful classification efficacy. Furthermore, the predicted effects of quetiapine treatment in BD patients were also assessed and achieved an AUC of 0.93 between responders and nonresponders. These findings provide important evidence that gut microbiota-targeted biomarkers were potentially helpful in predicting the treatment outcome of BD. Nevertheless, the microbial marker-based models still need to be validated in larger samples.

Several limitations of this study should be mentioned. Gut microbiota can be influenced by multiple variables. Although use of antibiotics, probiotics, and prebiotics was not allowed in our study, participants did not receive a standardized diet. Regional variation could also be an influential factor of gut microbiota.\textsuperscript{[38]} However, the regional sources of our samples were not controlled. Of note, the age of participants was not matched between patients and controls. Although we found a weak association of age with gut microbial composition, the effect of cohort was comparatively more significant. Another limitation was the follow-up rate in our patients, resulting in re-examining only a subset of patients. Although this study evaluated the impact of quetiapine monotherapy on the gut ecosystem, patients were only assessed at two time points. Therefore, our study cannot explain whether the changes in microbiota are trait- or state-related feature. Longitudinal design with patients in mania or remission is needed to clarify the cause–effect relationship between BD and gut microbiota.

4. Conclusions

This study indicated significant alterations in gut microbiota in depressed drug-free BD patients, which may be related to multiple factors, including metabolic pathways, depressive severity, and pharmacological treatment. Short-term quetiapine treatment failed to draw the microbial ecosystem of BD patients close to that of healthy individuals. Notably, gut microbial markers might be helpful for classifying BD and predicting treatment outcome. These findings provide further evidence that the microbiota–gut–brain axis is involved in BD pathogenesis. Future investigations should dig deeper to clarify the connections between gut microbiota and brain function in BD patients.

5. Experimental Section

\textbf{Participants:} This study was approved by the Institutional Review Board of the First Affiliated Hospital, School of Medicine of Zhejiang University (reference number #2017-397), and performed in accordance with the Helsinki Declaration. Written informed consent was obtained from all participants. BD patients with a current depressive episode were recruited from the Psychiatric Department of the First Affiliated Hospital, School of Medicine, Zhejiang University. Diagnoses according to DSM-IV-TR criteria for BD-I, BD-II and BD not
were annotated using RDP classifier version 2.12 [47] with a significance "global" function with 97% cutoff (-id 0.97). All OTU sequences were usearch with representative OTU sequences as reference using the "Usearch profiles were constructed by aligning randomly selected sequences into different samples: 1) no mismatch in primers and barcode regions; 2) a maximum mismatch rate in the overlapping region no more than 0.05; 3) maximum mismatch bases in the overlapping region no more than 5; 4) no ambiguous bases allowed in all reads; 5) minimum and maximum lengths of read (without barcodes and primers) of 100 bp and 550 bp, respectively. Next, chimeras were removed by using Uchime[45] with the "-strand plus" parameter and the recommended built-in sequence (http://drive5.com/uchime.gold.fa) as reference. Filtered reads were used for downstream analysis.

**OTU and KEGG Function Module Profiles:** Randomly selected sequences from all samples were clustered into OTUs using the "Usearch -cluster_otus" function[46] with default parameters (unique reads with abundance no more than two were filtered out). Then, OTU profiles were constructed by aligning randomly selected sequences with representative OTU sequences as reference using the "Usearch -usearch_global" function with 97% cutoff (-id 0.97). All OTU sequences were annotated using RDP classifier version 2.12[47] with a significance level of 0.80 (-c 0.8 -f fixrank); sequences which could not be assigned into a specific taxonomy level were labeled as "unclassified." PICRUSt version 1.0.0[48] was used to generate KEGG ontology profile. Human version 0.99[49] was used to assign ontology into the KEGG pathway at the module level.

**Alpha Diversity and Distance Calculation:** Relative abundance in OTU profiles was used to determine alpha diversity and distance matrix for all samples. The Vegan version 2.4.4[50] package was used to determine Shannon, Simpson, and invSimpson indexes. The Simpson’s index in this study was a variant of the original Simpson’s diversity index (D), and obtained as 1-D. The number of OTUs in each sample was calculated as Obs index. The Fossil[51] version 0.3.7 package was used to determine Chao 1 and ICE index. A custom R script (http://enterotyping.embl.de/enterotypes.html) was used to determine JSD. Spearman coefficient distance was calculated in the R program as follows: 1- dist(cor(dat), method = "spearman"), where dat is the relative abundance of the OTU profile. MUSCLE v3.8.3[52] was used to perform multiple alignments of representative OTU sequences, with FastTree version 2.1.8[53] employed to generate a phylogenetic tree with the generalized time-reversible model. The phyloseq package version 1.20.0[54] was used to obtain the UniFrac distance (weighted and unweighted), with relative OTU abundance and rooted phylogenetic tree. To explore whether differences of gut microbiota exist between different types of BD, we use PCoA to calculate the beta diversity by weighted UniFrac, unweighted UniFrac, hellinger and JSD.

**Statistical Analysis:** Wilcoxon rank sum test was used to perform hypothesis test of alpha diversity and PCs among different cohorts. Lefse version 1.0[55] was used to identify taxa and KEGG modules, which may be significantly associated with quetiapine treatment or cohorts (LDA score (log10) ≥ 2 as cutoff value). For paired samples (two samples before and after quetiapine treatment, from the same patients), p-values by paired Wilcoxon rank sum test were used, instead of Lefse build-in hypothesis test. Permutational ANOVA test[56] was employed to determine the significance of difference between health and BD patient groups (i.e., HC vs BD; BD pretreatment vs BD posttreatment; BD-I vs BD-II). Statistical significance of each test was determined by comparing the actual F test result from ANOVA to that obtained from 10 000 random permutations of the individuals between the compared groups of each test. All these analyzes were conducted with R package.[57] Spearman’s rho statistic was used to estimate the association between clinical parameters and taxa.

**Random Forest Classification Models:** Random Forest 4.6-12[58] was used to build classification models using profiles of genera with significant differences between the two cohorts. The term "POBD" was used as samples with probabilities could be classified as “BD.” The LOO cross validation mode was used; thus, in the training stage, one in all m samples would be trained in m training models. Finally, mean POBD was used as the final training POBD. The evaluation of the random forest classification models was performed by ROC curve (R 3.3.0, PROC package), and AUC was used to assess the ROC effect. The same method was used to predict the treatment outcome in BD patients.

**Supporting Information** Supporting Information is available from the Wiley Online Library or from the author.

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**Conflict of Interest** The authors declare no conflict of interest.
