



## Differences in gut microbiome composition between persons with chronic schizophrenia and healthy comparison subjects

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### ABSTRACT

Intestinal microbiome and gut-brain axis have been receiving increasing attention for their role in the regulation of brain/behavior and possible biological basis of psychiatric disorders. Several recent clinical studies have linked the microbiome with neuropsychiatric conditions, although the literature on schizophrenia is quite limited. This study investigated gut microbiome composition in 50 individuals, including 25 persons with chronic schizophrenia and 25 demographically-matched non-psychiatric comparison subjects (NCs). Stool samples were collected and assayed using 16S rRNA sequencing of the V4 region. Examination of unweighted UniFrac and Bray-Curtis dissimilarity revealed significant community-level separation in microbiome composition between the two subject groups. At the phylum level, *Proteobacteria* were found to be relatively decreased in schizophrenia subjects compared to NCs. At the genus level, *Anaerococcus* was relatively increased in schizophrenia while *Haemophilus*, *Sutterella*, and *Clostridium* were decreased. Within individuals with schizophrenia, abundance of *Ruminococcaceae* was correlated with lower severity of negative symptoms; *Bacteroides* was associated with worse depressive symptoms; and *Coprococcus* was related to greater risk for developing coronary heart disease. Our findings provide evidence of altered gut microbial composition in persons with chronic schizophrenia and suggest a need for larger and longitudinal studies of microbiome in schizophrenia.

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### 1. Introduction

Recent years have seen a rapid growth of interest in the human gut microbiome in the pathophysiology of human health and disease. The microbiome is a dynamic ecological community of microorganisms that inhabit the human body. The gut microbiome has a pivotal role in regulating inflammatory and metabolic pathways across a number of physical diseases, including gastrointestinal (GI) disorders, such as inflammatory bowel disease (Kostic et al., 2014), obesity and metabolic diseases (Bouter et al., 2017; Hartstra et al., 2015), cancer (Schwabe and Jobin, 2013), and chronic pulmonary diseases (Budden et al., 2017; O'Dwyer et al., 2016). There is also an emerging body of empirical support for the view that the gut microbiome can impact brain and behavior (Collins et al., 2012; Cryan and Dinan, 2012; Mayer, 2011). A

number of animal studies suggest that the alterations in gut microbiota can induce physiological and behavioral features that resemble psychiatric disorders (e.g., Sudo et al., 2004; Zheng et al., 2016). However, human studies in this area have been few, particularly in schizophrenia (Nguyen et al., 2018).

Converging evidence suggests that schizophrenia and other serious mental illnesses are associated with chronic systemic and gastrointestinal inflammation, oxidative stress, and metabolic dysfunction (Nguyen et al., 2018). Some studies have found that schizophrenia is associated with elevated serological biomarkers of microbial translocation (Severance et al., 2012; Severance et al., 2016; Severance et al., 2013), suggesting increased permeability of the intestinal lumen, which may be a mechanism by which intestinal dysbiosis impacts systemic physiological functioning. Additionally, gut and digestive disturbances, including irritable bowel syndrome, colitis, and celiac disease, are highly prevalent comorbidities in persons with schizophrenia (Severance et al., 2015). Gastrointestinal diseases are the third leading cause of natural deaths in schizophrenia (Saha et al., 2007). Considering that microbial colonization of the gut is crucial for optimal function of the immune

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system (Kamada et al., 2013; Round and Mazmanian, 2009), inflammation, oxidative stress, and other physiological dysfunctions implicated in schizophrenia might be, at least in part, associated with changes in the microbiome.

The number of empirical studies of the gut microbiome in persons with schizophrenia is very limited (Nguyen et al., 2018). The four published studies of the gut microbiome in psychotic disorders include a study of individuals at high-risk and ultra-high-risk for schizophrenia (He et al., 2018), two investigations of patients with first-episode psychosis (Schwarz et al., 2018; Yuan et al., 2018), and a report on persons with schizophrenia of <10 years' duration (Shen et al., 2018). These studies revealed that the species composition within the gut microbiomes of individual with or at risk for psychotic disorders is different from that of non-psychiatric comparison subjects (NCs), with varying bacterial taxa driving community separation in each study. Only one of these studies investigated the relationship between gut bacteria and clinical characteristics; first-episode patients with the greatest abnormalities in microbiota composition had more severe psychotic symptoms and worse global functioning at hospitalization and a lower rate of disease remission at one-year follow-up (Schwarz et al., 2018).

These studies focused on psychosis at earlier stages in the disease process and were conducted on European and Chinese subjects. Microbial composition varies across individuals with different countries of residence (McDonald et al., 2018). One study (Shen et al., 2018) excluded individuals with any chronic disease that may affect the stability of gut microbiota, including hypertension and diabetes, which are common in schizophrenia. To our knowledge, the present report is the first investigation of gut microbiome in US-based patients with chronic schizophrenia.

Our objective was to characterize gut microbiome in patients with schizophrenia, with duration of >10 years (range 12–56 years). We hypothesized that gut microbial composition would differ between schizophrenia subjects and NCs. We also sought to explore relationships of microbial composition differences with clinical and disease characteristics, specifically with psychiatric symptomatology, smoking, physical comorbidity, and antipsychotic medications.

## 2. Methods

### 2.1. Participants

Fifty subjects, including 25 outpatients with schizophrenia or schizoaffective disorder (hereafter referred to collectively as schizophrenia) and 25 demographically matched NCs, between the ages of 30 and 76 years, participated in this study. The diagnosis of schizophrenia was made based on the Structured Clinical Interview for the DSM-IV-TR (SCID) (First et al., 2002). Twenty-one patients were on antipsychotic medication at the time of the study. NC participants were recruited through multiple methods, including from an ongoing survey study of successful aging in healthy adults at UCSD, recruitment flyers in the community, [www.ResearchMatch.org](http://www.ResearchMatch.org), and word-of-mouth. See Hong et al. (2017), Joseph et al. (2015), and Lee et al. (2016, 2017) for additional details regarding recruitment and subject selection criteria. NCs were screened using the Mini-International Neuropsychiatric Interview and excluded from the study if they had a past or present diagnosis of a major neuropsychiatric illnesses (Sheehan et al., 1998). Exclusion criteria were: other current major DSM-IV-TR Axis I diagnoses; alcohol or other substance (other than tobacco) abuse or dependence within 3 months prior to enrollment; diagnosis of dementia, intellectual disability disorder, or a major neurological disorder; or any medical disability that interfered with a subject's ability to complete study procedures. We did not exclude persons with chronic diseases such as hypertension and diabetes. Although these conditions may influence the stability of the gut microbiota, patients with schizophrenia tend to have higher rates of these chronic medical conditions (De Hert et al., 2009; Hennekens et al., 2005). All procedures were approved by the

UCSD Human Research Protections Program. All participants provided written informed consent.

Given that technical differences (e.g., DNA extraction methods, sequencing platforms, taxonomy databases) can produce systematic biases that may obscure biologically meaningful compositional differences (Lozupone et al., 2013; Walters et al., 2014), NC subjects were matched to schizophrenia subjects on sequencing plate after initial recruitment. We found the nearest matching neighbors on the sequencing plate based on age, sex, race, BMI category (obese vs. not obese), and history of antibiotic use (in the past year) to control for clinical factors and known major drivers of microbiome changes (McDonald et al., 2018) that could confound the results.

### 2.2. Sociodemographic and clinical assessment

Sociodemographic characteristics (i.e., age, gender, race/ethnicity, current smoking status) and illness-related factors (i.e., age of onset, duration of illness, antipsychotic medication) were ascertained through participant interview and review of available medical records. Daily dosages of antipsychotic medication were converted to total World Health Organization defined daily dose (Organization, 2009). BMI was based on assessment of height and weight (kg/m<sup>2</sup>). Positive and negative psychiatric symptoms were evaluated using interviewer-administered Scales for Assessment of Positive Symptoms and Negative Symptoms (SAPS and SANS, respectively; Andreasen, 1983, 1984). Depression was assessed using the Patient Health Questionnaire (PHQ-9; Kroenke and Spitzer, 2002). Health-related quality of life and functioning was evaluated using the physical and mental health component scores from the Medical Outcomes Study 36-item Short Form (SF-36; Ware Jr and Sherbourne, 1992). Medical comorbidity was measured with the total score and severity index from the Cumulative Illness Rating Scale (CIRS; Parmelee et al., 1995). The Framingham 10-year Coronary Heart Disease (CHD) relative risk score was calculated according to Wilson et al. (1998), and Framingham 10-year Cardiovascular Disease (CVD) risk score according to D'Agostino Sr. et al. (2008).

### 2.3. Stool collection and sequencing

Participants were provided with home stool collection kits (BD SWUBE Dual Swab Collection System; BD Worldwide) and given detailed instructions about how to collect stool samples. Participants returned samples via mail in a self-addressed envelope. Returned stool samples were frozen at –80 °C until aliquoted into 96 well plates for DNA extraction.

DNA extraction, amplification, and sequencing were performed in the same way as for the American Gut Project samples (americangut.org; McDonald et al., 2018). Genomic DNA was extracted from samples using the Earth Microbiome 16S rRNA amplicon extraction protocol (Earth Microbiome Project, 2016). PCR amplification and library preparation were performed similarly to the protocol described by Caporaso et al. (2012). Illumina primers with unique reverse primer barcodes were used to target the V4 region of the 16S ribosomal RNA gene. V4 amplicons were sequenced on the Illumina HiSeq 2000 platform, yielding paired-end, 150-base-pair reads. Sequencing was performed at the UCSD IGM Genomics Center. Feature tables can be found in Qiita ([qiita.ucsd.edu](http://qiita.ucsd.edu)) as study ID 11710 and sequences can be found in EBI-ENA under accession number EBI: ERP107975.

### 2.4. Bioinformatics and statistical analyses

Sequence data were processed using QIIME2 ([qiime2.org](http://qiime2.org); Caporaso et al., 2010). Raw sequencing results were demultiplexed and microbial sOTUs (sub-Operational Taxonomic Units) were identified using Deblur algorithm (Amir et al., 2017). The output feature table was rarefied to 7905 sequences per sample (the lowest value in the dataset). Microbial community structure was characterized using measures of alpha-

diversity (within-sample) and beta-diversity (between-samples). Alpha-diversity represents the number (richness) and distribution (evenness) of taxa within a single sample. Metrics of alpha-diversity included number of observed features to represent species richness, Shannon diversity index to quantify species abundance and evenness (Shannon, 1948), and Faith's Phylogenetic Diversity which measures the total length of branches in a reference phylogenetic tree for all species in a given sample (Faith, 1992). Beta-diversity indicates differences in taxa composition between samples based on either presence-absence or quantitative species abundance data, and may be presented in a distance matrix. Beta-diversities were calculated using Bray-Curtis dissimilarity (Sørensen, 1948) and unweighted UniFrac (Lozupone and Knight, 2005; Lozupone et al., 2007). Output matrices were ordinated using principal coordinate analysis (PCoA) and visualized using EMPeror (Vazquez-Baeza et al., 2013).

#### 2.4.1. Data analysis

Statistical significance of beta-diversity distances between groups was assessed using PERMANOVA with 999 permutations. Alpha-diversity group significance was calculated using nonparametric Kruskal-Wallis  $H$  test in QIIME2. For taxonomic analyses and differential abundance testing, taxonomy information was provided for each sOTU sequence using UCLUST method implemented in QIIME1 (Caporaso et al., 2010; Edgar, 2010). All differential abundance testing, at different taxonomic levels were performed using Calour ([github.com/biocore/calour](https://github.com/biocore/calour)) with mean difference permutative test (equivalent to Kruskal-Wallis  $H$  test in the case of 2 groups) at 10,000 permutations with discrete False Discovery Rate (FDR) correction at  $\alpha = 0.1$  (Jiang et al., 2017). To determine associations between specific taxa and clinical characteristics, data were pre-processed in the same way as for differential abundance testing (filtering features with <1000 reads, normalizing data to 10,000 reads and clustering features with <100 reads together). Pearson's correlations were then calculated between each sOTU (feature) and demographic and/or clinical characteristics within schizophrenia and NC groups separately; a  $t$ -statistic test using 1000 permutations (where labels are randomly changed between samples) with discrete FDR correction was applied to determine the statistical significance of features (Jiang et al., 2017).

### 3. Results

Demographic and clinical characteristics for groups are presented in Table 1. Schizophrenia and NC groups did not differ on age, gender, or race. As expected, persons with schizophrenia had worse psychiatric symptoms, higher depression and anxiety levels, lower levels of physical well-being, higher rates of smoking, and greater medical comorbidity. Comparing exact BMI values, persons with schizophrenia had higher BMI than NCs; however, in terms of BMI classification, there were no significant differences between the two groups.

#### 3.1. Microbial community structure

There was no difference between schizophrenia and NC groups on any assessed measure of alpha-diversity (Supplemental Table 1). Analysis of beta-diversity indices revealed significant community-level separation between schizophrenia and NC groups using unweighted UniFrac (pseudo- $F = 1.81$ ,  $p = 0.005$ ) and Bray-Curtis dissimilarity (pseudo- $F = 2.20$ ,  $p = 0.003$ ). PCoA of unweighted UniFrac and Bray-Curtis distances showed that schizophrenia and NC groups formed distinct clusters (Fig. 1). NCs also clustered more tightly than schizophrenia subjects, who were spread more widely across the PCoA space (within-group distance comparison with a two-sided  $t$ -test; unweighted UniFrac  $p < 0.001$ ; Bray-Curtis  $p = 0.02$ ). Across groups, significant beta-diversity differences were also observed for sex (unweighted UniFrac pseudo- $F = 1.66$ ,  $p = 0.007$ ) and smoking status (unweighted UniFrac pseudo- $F = 1.43$ ,  $p = 0.027$ ; Bray-Curtis pseudo- $F$

**Table 1**  
Demographic and clinical characteristics of participants.

	Schizophrenia ( $n = 25$ )	Non-psychiatric comparison ( $n = 25$ )	$t$ or $\chi^2$	$p$
Age (years)	52.9 (11.2)	54.7 (10.7)	0.58	0.56
Gender [ $n$ (% female)]	11 (44%)	10 (40%)	0.08	0.77
Race [ $n$ (% Caucasian)]	16 (64%)	19 (76%)	1.07	0.79
BMI	31.8 (5.4)	28.9 (4.0)	-2.17	0.04
BMI classification <sup>b</sup> [ $n$ (% obese)]	16 (64%)	10 (40%)	2.89 <sup>a</sup>	0.09
Antibiotic use [ $n$ (% in past year)]	7 (28%)	5 (20%)	0.44 <sup>a</sup>	0.51
Current smoking status [ $n$ (% smoker)]	14 (56%)	1 (4%)	16.1 <sup>a</sup>	<0.001
Smoking frequency	Daily: 12 Regularly <sup>d</sup> : 1 Rarely <sup>e</sup> : 1 Never: 11	Daily: 1 Never: 24	16.1	0.001
Age of illness onset (years)	21.5 (7.0)	-	-	-
Illness duration (years)	32.4 (11.1)	-	-	-
Antipsychotic daily dosage <sup>e</sup>	2.01 (2.6)	-	-	-
SAPS Total Score	4.56 (3.3)	0.67 (0.8)	-5.31	<0.001
SANS Total Score	4.16 (5.1)	0.50 (0.8)	-1.73	0.10
PHQ-9 Severity Score	7.21 (6.1)	3.20 (2.3)	-2.96	0.006
BSI Anxiety Score	8.25 (6.1)	2.00 (3.0)	-4.43	<0.001
SF-36 Mental Component	43.5 (12.9)	51.6 (8.3)	2.40	0.2
SF-36 Physical Component	43.3 (11.6)	52.8 (7.5)	3.13	0.003
CIRS Total Score	6.6 (2.8)	1.5 (2.0)	-4.24	<0.001
CIRS Severity Score	1.57 (0.3)	0.72 (0.9)	-2.39	0.06
Framingham CHD risk score	1.69 (1.0)	0.94 (0.13)	-3.15	0.005
Framingham CVD risk score	19.1 (15.3)	6.53 (2.28)	-3.48	0.002
Medical diagnoses				
Diabetes [ $n$ (% with)]	9 (36%)	1 (4%)	8.0 <sup>a</sup>	0.005
Hypertension [ $n$ (% with)]	19 (76%)	2 (8%)	4.0 <sup>a</sup>	0.05

BMI = body mass index; BSI = Brief Symptom Inventory; CHD = coronary heart disease; CVD = cardiovascular disease; PHQ-9 = Patient Health Questionnaire; SANS = Scale for the Assessment of Negative Symptoms; SAPS = Scale for the Assessment of Positive Symptoms.

<sup>a</sup>  $\chi^2$  value.

<sup>b</sup> World Health Organization classification; comparison of obese (BMI  $\geq 30$ ) to non-obese (BMI < 30) individuals.

= 1.55,  $p = 0.042$ ), the latter of which was likely driven by diagnosis, as all but one smoker were schizophrenia subjects. Microbial community separation related to diagnostic group demonstrated the highest effect size (pseudo- $F = 1.81$ ).

#### 3.2. Bacterial taxonomic composition

Differential abundance testing at different taxonomic levels was performed to identify the drivers of community separation. At the phylum level, *Proteobacteria* was observed to be differentially abundant between groups, with schizophrenia subjects having decreased relative abundance of this phylum compared to NCs (Supplemental Fig. 1). At the genus level, four differentially abundant genera were revealed. *Anaerococcus* was relatively increased in schizophrenia compared to NCs ( $H = 8.32$ ;  $p = 0.007$ ), while *Haemophilus* ( $H = -11.3$ ;  $p = 0.004$ ), *Sutterella* ( $H = -12.0$ ;  $p = 0.004$ ), and *Clostridium* ( $H = -15.9$ ;  $p = 0.0002$ ) were decreased (Fig. 2). When differential abundance testing was performed across taxonomic levels, 35 individual taxa were found to be differentially abundant between schizophrenia subjects and NCs. Thirty-three sOTUs belonged to the order *Clostridiales*, 1 sOTU to class *Gammaproteobacteria* (*Haemophilus parainfluenzae*) and 1 sOTU to class *Erysipelotrichi* (unknown genus) (Supplemental Table 2).

### 3.3. Relationship with clinical characteristics

Age was not significantly associated with alpha-diversity (species richness, abundance, or evenness) across the entire sample ( $r_s < 0.16$ ;  $p_s > 0.29$ ) or within either schizophrenia ( $r_s < 0.04$ ;  $p_s > 0.86$ ) or NC ( $r_s < 0.35$ ;  $p_s > 0.08$ ) groups. Within schizophrenia subjects, age of disease onset was positively correlated with phylum *Cyanobacteria* ( $r = 0.53$ ;  $p = 0.008$ ), such that persons with earlier onset of illness had lower abundance of this taxa. No relationship was found between illness duration and specific taxa.

Specific microbial taxa were associated with level of psychopathology in schizophrenia. Greater severity of depressive symptoms was correlated with greater abundance of genus *Bacteroides* ( $r = 0.70$ ;  $p = 0.0002$ ), while increased negative symptoms were associated with decreased abundance of family *Ruminococcaceae* ( $r = -0.74$ ;  $p = 0.0002$ ). Overall self-reported mental well-being was positively correlated with phylum *Verrucomicrobia* ( $r = 0.63$ ;  $p = 0.002$ ). Higher level of the genus *Coprococcus* was associated with greater Framingham

CHD risk ( $r = 0.77$ ;  $p = 0.0003$ ). Schizophrenia patients with and without diabetes (unweighted UniFrac pseudo- $F = 1.21$ ,  $p = 0.11$ ; Bray-Curtis pseudo- $F = 1.03$ ,  $p = 0.39$ ), hypertension (unweighted UniFrac pseudo- $F = 0.82$ ,  $p = 0.86$ ; Bray-Curtis pseudo- $F = 1.04$ ,  $p = 0.38$ ), or heart disease (unweighted UniFrac pseudo- $F = 1.00$ ,  $p = 0.41$ ; Bray-Curtis pseudo- $F = 1.15$ ,  $p = 0.26$ ) did not differ in gut microbial composition.

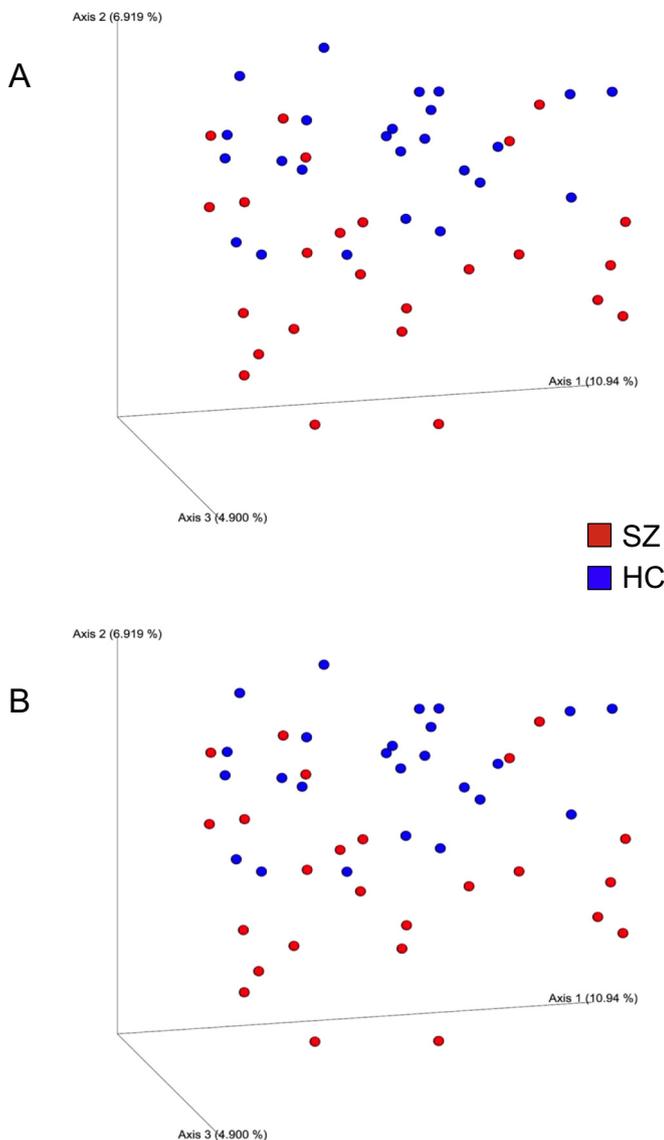
To disentangle the effects of smoking and diagnosis, we compared smokers ( $n = 14$ ) to non-smokers ( $n = 11$ ) within the schizophrenia group and found no difference in beta-diversity (unweighted UniFrac pseudo- $F = 1.26$ ,  $p = 0.085$ ; Bray-Curtis pseudo- $F = 0.94$ ,  $p = 0.485$ ), suggesting that differences in microbial community composition between study groups are driven by disease rather than cigarette smoking. Within the schizophrenia group, greater number of years of smoking was associated with higher levels of phylum *Actinobacteria* ( $r = 0.48$ ;  $p = 0.01$ ). We found no significant associations between specific microbial taxa and antipsychotic daily dosage levels ( $r_s < 0.09$ ;  $p_s > 0.66$ ).

### 4. Discussion

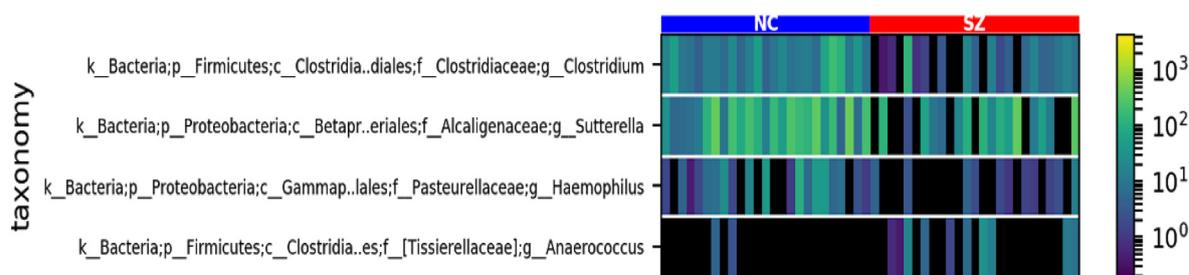
Consistent with our hypothesis, this study found several significant differences in the overall composition and levels of specific bacterial taxa in the gut microbiome in persons with schizophrenia compared to NCs. To our knowledge, this investigation is the first to provide evidence for altered gut microbiota in a sample of chronically ill patients with schizophrenia living in the US. Notably, most of these differences remained significant after controlling for demographic and clinical factors that may influence microbial composition. A number of bacterial taxa were differentially abundant between groups, including decreased relative abundance of phylum *Proteobacteria* and genera *Haemophilus*, *Sutterella* and *Clostridium* and increased relative abundance of genus *Anaerococcus* in schizophrenia compared to NCs. The finding of abnormal microbial composition in patients with schizophrenia is consistent with previous reports among other schizophrenia-spectrum (He et al., 2018; Shen et al., 2018) and first-episode psychotic (Yuan et al., 2018) disorders, although the specifics of the observed differences vary.

When considering the relative abundance of specific taxa, findings have been mixed across studies. We found that abundance of *Proteobacteria* and *Clostridium* was relatively lower in schizophrenia patients than NCs, which was opposite to findings reported by Shen et al. (2018). Inconsistencies across investigations may be attributable partly to heterogeneity in sample characteristics across studies. Each of the prior four studies focused on schizophrenia and psychotic disorders and different developmental stages (He et al., 2018; Schwarz et al., 2018; Shen et al., 2018; Yuan et al., 2018). Furthermore, we did not exclude individuals with certain chronic diseases, as these bodily and physiological changes are inherent to the disorder and associated lifestyle. Similar to all previous studies, patients with schizophrenia and NCs did not differ in terms of within-sample (alpha) diversity, despite overall between-sample composition divergence between patients and NCs.

Finally, our study revealed some potentially interesting information about the relationship between the gut microbiome and clinical characteristics associated with schizophrenia. We found that the composition of the gut microbiome was associated with psychopathology. Increased *Ruminococcaceae* was correlated with decreased negative symptoms, and *Bacteroides* with worse depressive symptoms. Family *Ruminococcaceae* has been previously reported in the literature to be associated with major depressive disorder (Jiang et al., 2015; Zheng et al., 2016) and bipolar disorder (Evans et al., 2016). Studies have generally found this family to be decreased in serious mental illnesses (Evans et al., 2016; Jiang et al., 2015), although it was reported to be overrepresented in one study (Zheng et al., 2016). Furthermore, higher levels of this taxon have been shown to be associated with decreased anxiety and depression and better sleep and physical well-being (Evans et al.,



**Fig. 1.** Principal coordinates analysis (PCoA) plot illustrating beta-diversity distance matrices of unweighted UniFrac distance (A) and Bray-Curtis distance (B) comparing sample distributions between schizophrenia and NC groups. Red dots represent schizophrenia patients, and blue dots represent NCs.



**Fig. 2.** Heat map illustrating differentially abundant genera in the two groups. Each vertical color bar represents an individual subject. The color of the bar indicates the relative abundance of each genus within a subject. The vertical color bar on the right side of the graph delineates relative abundance, and the horizontal bar at the top of the graph denotes schizophrenia and NC groups.

2016). The literature on the relationship between genus *Bacteroides* is mixed; it has been previously found to be relatively underrepresented in patients with active depression, yet overrepresented in patients with antidepressant-responsive depression (Jiang et al., 2015).

With regards to physical health, *Coprococcus* was associated with greater risk for developing CHD in patients with schizophrenia. This genus has been found to be enriched among individuals with a high lifetime cardiovascular risk profile (Kelly et al., 2016) and decreased following bariatric surgery in obese patients with diabetes (Graessler et al., 2013), suggesting that it may be a marker of vascular health. Comorbid diagnoses of diabetes, hypertension, or heart disease did not contribute significantly to microbiome differences in schizophrenia patients.

As expected, cigarette smoking was a significant driver of global microbial composition. To disentangle the effect of smoking from the effect of diagnosis in schizophrenia and NC groups, we examined patients who were smokers vs. non-smokers and found no differences in beta-diversity, suggesting that the microbial abnormalities in schizophrenia may not be primarily attributable to cigarette smoking. Increased number of years of smoking was associated with higher levels of *Actinobacteria*, consistent with previous studies showing that *Actinobacteria* is relatively enriched in current smokers compared to never smokers (Wu et al., 2016). The abundance of this phylum has also been found to increase after smoking cessation (Biedermann et al., 2013). Lastly, we did not detect any significant association between specific taxa and daily dosages of antipsychotics. Prior studies have evaluated the effects of antipsychotic medications on the gut microbiome in serious mental illnesses. In drug-naïve patients with first episode schizophrenia, 24-week risperidone treatment resulted in significant increases in the numbers of *Bifidobacterium* spp. and *E. coli*, and decreases in *Clostridium coccoides* group and *Lactobacillus* spp. (Yuan et al., 2018). Current atypical antipsychotic treatment was also associated with reduced gut alpha-diversity in women with bipolar disorder and significant beta-diversity separation (Flowers et al., 2016). Patients on atypical antipsychotic medications demonstrated relatively increased levels of *Lachnospiraceae*, while non-antipsychotic-treated individuals showed preferentially higher levels of *Akkermansia*.

#### 4.1. Limitations

Several limitations to our study must be acknowledged. The sample size in this investigation was small, limiting the extent to which we could statistically explore potential confounds on gut microbial differences and the power of correlational analyses to assess the relationship of various clinical correlates and gut microbiome. Thus, these results should be considered preliminary. We were able to replicate community-level group differences found in other studies of the gut microbiome in populations with or at risk for serious mental illnesses (Evans et al., 2016; He et al., 2018; Schwarz et al., 2018; Shen et al., 2018), and we observed several intriguing relationships that should be further explored and replicated in future studies. Future investigations should aim to further assess the relationship of psychotropic medication

use as well as disease severity, cognition, nutrition, and lifestyle behaviors to the gut microbiome. It is also a possibility that microbial differences between schizophrenia patients and NCs may be driven by the larger percentage of patients who have co-morbid medical illnesses (e.g., diabetes, hypertension). However, we did not find any beta-diversity differences between patients with and without these disorders, suggesting that these physical conditions did not significantly impact the differences observed between groups. Finally, the cross-sectional design restricts our ability to make causal inferences about the role of the gut microbiome in schizophrenia. Larger, prospective longitudinal studies of patients with varied durations of illness are required to determine whether intestinal dysbiosis is a key pathophysiological feature of schizophrenia and how it may impact aging in this disorder.

Despite these limitations, this study possesses several strengths. We included a NC sample that was matched to the schizophrenia sample on demographics, sequencing plate, and other clinical factors known to have major influences on the gut microbiome. Our study is also the first to examine schizophrenia patients living in the US and those with very long durations of illness. These results represent a first step in understanding the gut microbial ecosystem in clinical populations of persons with schizophrenia. These findings lay the groundwork for future investigations in elucidating the role of the gut microbiome in the development, presentation, and progression of schizophrenia and how it may be associated with (or contribute to) other physiological abnormalities (e.g., chronic inflammation, oxidative stress) in this population. Microbiome research holds promise for predicting clinical prognosis, assessing risk for medical morbidity and mortality, and informing intervention development (e.g., probiotics, prebiotics, fecal transplantation) to improve patients' quality of life. Strategies for achieving and maintaining healthy microbiome may lead to numerous potential benefits, including improved physical and mental health, cognition, and consequently, everyday functioning.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.schres.2018.09.014>.

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### Conflict of interest

The authors have declared that there are no conflicts of interest in relation to the subject of this study.

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### References

- Amir, A., McDonald, D., Navas-Molina, J.A., Kopylova, E., Morton, J.T., Zech Xu, Z., Kightley, E.P., Thompson, L.R., Hyde, E.R., Gonzalez, A., Knight, R., 2017. Deblur Rapidly Resolves Single-Nucleotide Community Sequence Patterns. *2(2)*. mSystems.
- Andreasen, N.C., 1983. Scale for the Assessment of Negative Symptoms (SANS). University of Iowa, Iowa City, IA.
- Andreasen, N.C., 1984. Scale for the Assessment of Positive Symptoms (SAPS). University of Iowa, Iowa City, IA.
- Biedermann, L., Zeitz, J., Mwynyi, J., Sutter-Minder, E., Rehman, A., Ott, S.J., Steurer-Stey, C., Frei, A., Frei, P., Scharl, M., Loessner, M.J., Vavricka, S.R., Fried, M., Schreiber, S., Schuppler, M., Rogler, G., 2013. Smoking cessation induces profound changes in the composition of the intestinal microbiota in humans. *PLoS One* 8 (3), e59260.
- Bouter, K.E., van Raalte, D.H., Groen, A.K., Nieuwdorp, M., 2017. Role of the gut microbiome in the pathogenesis of obesity and obesity-related metabolic dysfunction. *Gastroenterology* 152 (7), 1671–1678.
- Budden, K.F., Gellatly, S.L., Wood, D.L.A., Cooper, M.A., Morrison, M., Hugenholtz, P., Hansbro, P.M., 2017. Emerging pathogenic links between microbiota and the gut-lung axis. *Nat. Rev. Microbiol.* 15 (1), 55–63.
- Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pena, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* 7 (5), 335–336.
- Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S.M., Betley, J., Fraser, L., Bauer, M., Gormley, N., Gilbert, J.A., Smith, G., Knight, R., 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME J.* 6 (8), 1621–1624.
- Collins, S.M., Surette, M., Bercik, P., 2012. The interplay between the intestinal microbiota and the brain. *Nat. Rev. Microbiol.* 10 (11), 735–742.
- Cryan, J.F., Dinan, T.G., 2012. Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nat. Rev. Neurosci.* 13 (10), 701–712.
- D'Agostino Sr., R.B., Vasan, R.S., Pencina, M.J., Wolf, P.A., Cobain, M., Massaro, J.M., Kannel, W.B., 2008. General cardiovascular risk profile for use in primary care: the Framingham Heart Study. *Circulation* 117 (6), 743–753.
- De Hert, M., Dekker, J.M., Wood, D., Kahl, K.G., Holt, R.I., Moller, H.J., 2009. Cardiovascular disease and diabetes in people with severe mental illness position statement from the European Psychiatric Association (EPA), supported by the European Association for the Study of Diabetes (EASD) and the European Society of Cardiology (ESC). *Eur. Psychiatry* 24 (6), 412–424.
- Earth Microbiome Project, 2016. DNA Extraction Protocol.
- Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 26 (19), 2460–2461.
- Evans, S.J., Bassis, C.M., Hein, R., Assari, S., Flowers, S.A., Kelly, M.B., Young, V.B., Ellingrod, V.E., McInnis, M.G., 2016. The gut microbiome composition associates with bipolar disorder and illness severity. *J. Psychiatr. Res.* 87, 23–29.
- Faith, D.P., 1992. Conservation evaluation and phylogenetic diversity. *Biol. Conserv.* 61 (1), 1–10.
- First, M., Spitzer, R.L., Gibbon, M., Williams, J.B., 2002. Structured Clinical Interview for DSM-IV-TR Axis I Disorders, Research Version, Patient Edition with Psychotic Screen (SCID-I/PW/PSY SCREEN) New York: Biometrics Research, New York State Psychiatric Institute; 2002. Biometrics Research, New York State Psychiatric Institute, New York.
- Flowers, S.A., Evans, S.J., Ward, K.M., McInnis, M.G., Ellingrod, V.L., 2016. Interaction between atypical antipsychotics and the gut microbiome in a bipolar disease cohort. *Pharmacotherapy* 37 (3), 261–267 (Epub 30 December 2016).
- Graessler, J., Qin, Y., Zhong, H., Zhang, J., Licinio, J., Wong, M.L., Xu, A., Chavakis, T., Bornstein, A.B., Ehrhart-Bornstein, M., Lamounier-Zepter, V., Lohmann, T., Wolf, T., Bornstein, S.R., 2013. Metagenomic sequencing of the human gut microbiome before and after bariatric surgery in obese patients with type 2 diabetes: correlation with inflammatory and metabolic parameters. *Pharmacogenomics J.* 13 (6), 514–522.
- Hartstra, A.V., Bouter, K.E.C., Backhed, F., Nieuwdorp, M., 2015. Insights into the role of the microbiome in obesity and type 2 diabetes. *Diabetes Care* 38 (1), 159–165.
- He, Y., Kosciolk, T., Tang, J., Zhou, Y., Li, Z., Ma, X., Zhu, Q., Yuan, N., Yuan, L., Li, C., Jin, K., Knight, R., Tsuang, M.T., Chen, X., 2018. Gut microbiome and magnetic resonance spectroscopy study of subjects at ultra-high risk for psychosis may support the membrane hypothesis. *Eur. Psychiatry* 53, 37–45.
- Hennekens, C.H., Hennekens, A.R., Hollar, D., Casey, D.E., 2005. Schizophrenia and increased risks of cardiovascular disease. *Am. Heart J.* 150 (6), 1115–1121.
- Hong, S., Lee, E.E., Martin, A.S., Soontornniyomkij, B., Soontornniyomkij, V., Achim, C.L., Reuter, C., Irwin, M.R., Eyster, L.T., Jeste, D.V., 2017. Abnormalities in chemokine levels in schizophrenia and their clinical correlates. *Schizophr. Res.* 181, 63–69.
- Jiang, L., Ling, Z., Zhang, Y., Mao, H., Ma, Z., Yin, Y., Wang, W., Tang, W., Tan, Z., Shi, J., Li, L., Ruan, B., 2015. Altered fecal microbiota composition in patients with major depressive disorder. *Brain Behav. Immun.* 48, 186–194.
- Jiang, L., Amir, A., Morton, J.T., Heller, R., Arias-Castro, E., Knight, R., 2017. Discrete False-discovery Rate Improves Identification of Differentially Abundant Microbes. *2(6)*. mSystems.
- Joseph, J., Depp, C., Martin, A.S., Daly, R.E., Glorioso, D.K., Palmer, B.W., Jeste, D.V., 2015. Associations of high sensitivity C-reactive protein levels in schizophrenia and comparison groups. *Schizophr. Res.* 168 (1–2), 456–460.
- Kamada, N., Seo, S.U., Chen, G.Y., Nunez, G., 2013. Role of the gut microbiota in immunity and inflammatory disease. *Nat. Rev. Immunol.* 13 (5), 321–335.
- Kelly, T.N., Bazzano, L.A., Ajami, N.J., He, H., Zhao, J., Petrosino, J.F., Correa, A., He, J., 2016. Gut microbiome associates with lifetime cardiovascular disease risk profile among Bogalusa heart study participants. *Circ. Res.* 119 (8), 956–964.
- Kostic, A.D., Xavier, R.J., Gevers, D., 2014. The microbiome in inflammatory bowel disease: current status and the future ahead. *Gastroenterology* 146 (6), 1489–1499.
- Kroenke, K., Spitzer, R.L., 2002. The PHQ-9: a new depression diagnostic and severity measure. *Psychiatr. Ann.* 32 (9), 509–515.
- Lee, E.E., Eyster, L.T., Wolkowitz, O.M., Martin, A.S., Reuter, C., Kraemer, H., Jeste, D.V., 2016. Elevated plasma F2-isoprostane levels in schizophrenia. *Schizophr. Res.* 176 (2–3), 320–326.
- Lee, E.E., Hong, S., Martin, A.S., Eyster, L.T., Jeste, D.V., 2017. Inflammation in schizophrenia: cytokine levels and their relationships to demographic and clinical variables. *Am. J. Geriatr. Psychiatry* 25 (1), 50–61.
- Lozupone, C., Knight, R., 2005. UniFrac: a new phylogenetic method for comparing microbial communities. *Appl. Environ. Microbiol.* 71 (12), 8228–8235.
- Lozupone, C.A., Hamady, M., Kelley, S.T., Knight, R., 2007. Quantitative and qualitative beta diversity measures lead to different insights into factors that structure microbial communities. *Appl. Environ. Microbiol.* 73 (5), 1576–1585.
- Lozupone, C.A., Stombaugh, J., Gonzalez, A., Ackermann, G., Wendel, D., Vazquez-Baeza, Y., Jansson, J.K., Gordon, J.I., Knight, R., 2013. Meta-analyses of studies of the human microbiota. *Genome Res.* 23 (10), 1704–1714.
- Mayer, E.A., 2011. Gut feelings: the emerging biology of gut-brain communication. *Nat. Rev. Neurosci.* 12 (8), 453–466.
- McDonald, D., Hyde, E., Debelius, J.W., Morton, J.T., Gonzalez, A., Ackermann, G., Aksenov, A.A., Behaz, B., Brennan, C., Chen, Y., Deright Goldasich, L., Dorrestein, P.C., Dunn, R.R., Fahimipour, A.K., Gaffney, J., Gilbert, J.A., Gogul, G., Green, J.L., Hugenholtz, P., Humphrey, G., Huttenhower, C., Jackson, M.A., Janssen, S., Jeste, D.V., Jiang, L., Kelley, S.T., Knights, D., Kosciolk, T., Ladau, J., Leach, J., Marotz, C., Meleshko, D., Melnik, A.V., Metcalf, J.L., Mohimani, H., Montassier, E., Navas-Molina, J., Nguyen, T.T., Peddada, S., Pevzner, P., Pollard, K.S., Rahnava, G., Robbins-Pianka, A., Sangwan, N., Shorestein, J., Smarr, L., Song, S.J., Spector, T., Swafford, A.D., Thackray, V.G., Thompson, L.R., Tripathi, A., Vazquez-Baeza, Y., Vrbnac, A., Wischmeyer, P., Wolfe, E., Zhu, Q., American Gut, C., Knight, R., 2018. American Gut: an Open Platform for Citizen Science Microbiome Research. *3(3)*. mSystems.
- Nguyen, T.T., Kosciolk, T., Eyster, L.T., Knight, R., Jeste, D.V., 2018. Overview and systematic review of studies of microbiome in schizophrenia and bipolar disorder. *J. Psychiatr. Res.* 88, 50–61.
- O'Dwyer, D.N., Dickson, R.P., Moore, B.B., 2016. The lung microbiome, immunity, and the pathogenesis of chronic lung disease. *J. Immunol.* 196 (12), 4839–4847.
- Organization, W.H., 2009. Guideline for ATC Classification and DDD Assignment, 2010, WHO Collaborating Centre for Drug Statistics Methodology, Oslo, Norway.
- Parmelee, P.A., Thuras, P.D., Katz, I.R., Lawton, M.P., 1995. Validation of the cumulative illness rating scale in a geriatric residential population. *J. Am. Geriatr. Soc.* 43 (2), 130–137.
- Round, J.L., Mazmanian, S.K., 2009. The gut microbiome shapes intestinal immune responses during health and disease. *Nat. Rev. Immunol.* 9 (5), 313–323.
- Saha, S., Chant, D., McGrath, J., 2007. A systematic review of mortality in schizophrenia: is the differential mortality gap worsening over time? *Arch. Gen. Psychiatry* 64 (10), 1123–1131.
- Schwabe, R.F., Jobin, C., 2013. The microbiome and cancer. *Nat. Rev. Cancer* 13 (11), 800–812.
- Schwarz, E., Maukonen, J., Hyytiäinen, T., Kiesepa, T., Oresic, M., Sabuncuyan, S., Mantere, O., Saarela, M., Yolken, R., Suvisaari, J., 2018. Analysis of microbiota in first episode psychosis identifies preliminary associations with symptom severity and treatment response. *Schizophr. Res.* 192, 398–403.
- Severance, E.G., Alaedini, A., Yang, S., Halling, M., Gressitt, K.L., Stallings, C.R., Origoni, A.E., Vaughan, C., Khushalani, S., Leweke, F.M., Dickerson, F.B., Yolken, R.H., 2012. Gastrointestinal inflammation and associated immune activation in schizophrenia. *Schizophr. Res.* 138 (1), 48–53.
- Severance, E.G., Gressitt, K.L., Stallings, C.R., Origoni, A.E., Khushalani, S., Leweke, F.M., Dickerson, F.B., Yolken, R.H., 2013. Discordant patterns of bacterial translocation markers and implications for innate immune imbalances in schizophrenia. *Schizophr. Res.* 148 (1–3), 130–137.
- Severance, E.G., Prandovszky, E., Castiglione, J., Yolken, R.H., 2015. Gastroenterology issues in schizophrenia: why the gut matters. *Curr. Psychiatry Rep.* 17 (5), 27.
- Severance, E.G., Gressitt, K.L., Stallings, C.R., Katsafanas, E., Schweinfurth, L.A., Savage, C.L., Adams, M.B., Sweeney, K.M., Origoni, A.E., Khushalani, S., Leweke, F.M., Dickerson, F.B., Yolken, R.H., 2016. Candida albicans exposures, sex specificity and cognitive deficits in schizophrenia and bipolar disorder. *NPJ Schizophr.* 2, 16018.
- Shannon, C.E., 1948. The mathematical theory of communication. *Bell Syst. Tech. J.* 27 (379–423), 623–656.
- Sheehan, D.V., Lecrubier, Y., Sheehan, K.H., Amorim, P., Janavs, J., Weiller, E., Hergueta, T., Baker, R., Dunbar, G.C., 1998. The mini-international neuropsychiatric interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J. Clin. Psychiatry* 59, 22–33.
- Shen, Y., Xu, J., Li, Z., Huang, Y., Yuan, Y., Wang, J., Zhang, M., Hu, S., Liang, Y., 2018. Analysis of gut microbiota diversity and auxiliary diagnosis as a biomarker in patients with schizophrenia: a cross-sectional study. *Schizophr. Res.* 197, 470–477.

- Sørensen, T., 1948. A method of establishing groups of equal amplitude in plant sociology based on similarity of species content and its application to analyses of the vegetation on Danish commons. *Biol. Skr.* 5, 1–34.
- Sudo, N., Chida, Y., Aiba, Y., Sonoda, J., Oyama, N., Yu, X.N., Kubo, C., Koga, Y., 2004. Post-natal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. *J. Physiol.* 558 (Pt 1), 263–275.
- Vazquez-Baeza, Y., Pirrung, M., Gonzalez, A., Knight, R., 2013. EMPor: a tool for visualizing high-throughput microbial community data. *Gigascience* 2 (1), 16.
- Walters, W.A., Xu, Z., Knight, R., 2014. Meta-analyses of human gut microbes associated with obesity and IBD. *FEBS Lett.* 588 (22), 4223–4233.
- Ware Jr., J.E., Sherbourne, C.D., 1992. The MOS 36-item short-form health survey (SF-36): I. Conceptual framework and item selection. *Med. Care* 473–483.
- Wilson, P.W., D'Agostino, R.B., Levy, D., Belanger, A.M., Silbershatz, H., Kannel, W.B., 1998. Prediction of coronary heart disease using risk factor categories. *Circulation* 97 (18), 1837–1847.
- Wu, J., Peters, B.A., Dominianni, C., Zhang, Y., Pei, Z., Yang, L., Ma, Y., Purdue, M.P., Jacobs, E.J., Gapstur, S.M., Li, H., Alekseyenko, A.V., Hayes, R.B., Ahn, J., 2016. Cigarette smoking and the oral microbiome in a large study of American adults. *ISME J.* 10 (10), 2435–2446.
- Yuan, X., Zhang, P., Wang, Y., Liu, Y., Li, X., Kumar, B.U., Hei, G., Lv, L., Huang, X.F., Fan, X., Song, X., 2018. Changes in metabolism and microbiota after 24-week risperidone treatment in drug naive, normal weight patients with first episode schizophrenia. *Schizophr. Res.* in press.
- Zheng, P., Zeng, B., Zhou, C., Liu, M., Fang, Z., Xu, X., Zeng, L., Chen, J., Fan, S., Du, X., Zhang, X., Yang, D., Yang, Y., Meng, H., Li, W., Melgiri, N.D., Licinio, J., Wei, H., Xie, P., 2016. Gut microbiome remodeling induces depressive-like behaviors through a pathway mediated by the host's metabolism. *Mol. Psychiatry* 21 (6), 786–796.