Journal of Affective Disorders Reports 4 (2021) 100081

Contents lists available at ScienceDirect

Journal of Affective Disorders Reports

journal homepage: www.elsevier.com/locate/jadr

Research Paper

Description of the fecal microbiota of siblings from Costa Rica with and without affective and psychotic disorders

Sophie Ouabbou[a,b,*], Ching Jian[a], Alejandro Ávila-Aguirre[b], Henriette Raventós[b,c], Anne Salonen[a]

[a] Human Microbiome Research Program, Faculty of Medicine, University of Helsinki, Helsinki, Finland
[b] Cellular and Molecular Biology Research Centre, University of Costa Rica, San José, Costa Rica
[c] Department of Genetics, School of Biology, University of Costa Rica, San José, Costa Rica

ARTICLE INFO

Keywords:
Microbiome
Mental disorders
Bipolar disorder
Psychosis
Depression
Mood disorders

ABSTRACT

The pathogenesis of mental disorders remains poorly understood and there are enormous challenges in the development of biomarkers and more effective therapeutic options.

A total of 13 pairs of 26 siblings discordant for mental disorders, affected and unaffected, were subjected to Best Estimate Diagnosis process and clinical assessments with respect to their diagnosis, current mental health status and disability, resilience, medication, lifestyle and diet. All participants were of Hispanic ethnicity and residents of the San José Greater Metropolitan Area. Many of the pairs of siblings shared households. Affected individuals displayed major depression, bipolar affective disorder, psychosis non-otherwise specified or schizoaffective disorder. In a subsequent analysis the affected individuals were further divided according to whether they exhibited psychotic features or not.

Fecal samples were collected for the analysis of the gut microbiota using 16S rRNA gene amplicon sequencing and imputed metagenomics that were compared within the sibling pairs. Based on beta-diversity analysis, the use of levothyroxine and irbesartan was identified and thus used as confounders. Decreased bacterial richness was observed among the affected participants. The relative abundances of several bacterial taxa showed significant differences, including families *Peptostreptococcaceae, Ruminococcaceae, Porphyromonadaceae*, and genera *Pseudomonas, Barnesiella, Odoribacter, Paludibacter, Lactococcus, Clostridium, Acidaminococcus* and *Haemophilus.*

The proportion of Proteobacteria (*Pseudomonas*) was significantly increased in the affected individuals, while the bacterial taxa associated to healthy phenotype, such as *Barnesiella* and *Ruminococcaceae,* were depleted in individuals affected with psychosis in comparison to those without psychosis. Based on prediction functions of the gut microbiota, significant alternations were found in the pathways associated with amino acid metabolism in affected individuals in comparison to the unaffected.

Our findings suggest the changes in gut microbiota composition and functionality are associated with mental health, its diagnostics, and therapeutics.

1. Introduction

The present study focuses on individuals with mood disorders or with psychosis. Psychosis is a clinical syndrome defined in the Diagnostic Statistical Manual V (DSM-V) by the presence of delusions, hallucinations, disorganized thinking, grossly disorganized or abnormal motor behavior and negative symptoms (American Psychiatric Association 2013). Examples of negative symptoms are diminished emotional expression, avolition, or social withdrawal (American Psychiatric Association 2013). This syndrome is a common manifestation in several psychiatric disorders such as major depressive disorder with psychosis, bipolar disorder type I with psychosis, schizoaffective disorder and schizophrenia (Arciniegas 2015). Most of these disorders are ranked among the top causes of years lived with disability worldwide (Global Burden of Disease Study 2015). Psychiatric disorders are of multifactorial etiology where both genetic and environmental factors have been implicated with a particular focus on a possible autoimmune pathophysiology (Girgis et al., 2014; Al-Diwani et al., 2017). In the history of psychiatry, it has long been known that psychiatric disorders “run in families” and early studies did indeed document the heritability of disorders such as

* Corresponding author at: Human Microbiome Research Program, Faculty of Medicine, University of Helsinki, Helsinki, Finland. Haartmaninkatu 3 (PO Box 21)
E-mail address: sophie.ou@pm.me (S. Ouabbou).

https://doi.org/10.1016/j.jadr.2021.100081
Received 28 December 2020; Received in revised form 3 January 2021; Accepted 11 January 2021
Available online 18 January 2021

schizophrenia or bipolar disorder (Sullivan et al., 2018). It is worth noting that there is a shared genetic background between several disorders such as major depressive disorder, schizophrenia and bipolar disorder (Anttila et al., 2018) and that clinical diagnoses do not reflect with exactitude the biological pathophysiology of each (Tamminga et al., 2017). Efforts directed toward defining clearer mechanisms that explain the pathophysiology of mental disorders, biologically based diagnoses and novel treatments are greatly needed (Tamminga et al., 2017).

The study of a multifactorial condition, like mental disorders, requires the investigation of both genetic and environmental contributors to the pathophysiology. The microbiota is metabolically comparable to an organ that is malleable and sensitive to both host (genetic) (Blekhman et al., 2015) and environmental (McDonald et al., 2018) cues. Microbes associated with the human body include eukaryotes, archaea, bacteria and viruses and their communities together with their genes are known as the human microbiome (Methé et al., 2012). The composition of its ecosystems has implications in health and disease (Knight et al., 2017). It has now been researched that among its functions there is the harvest of inaccessible nutrients and synthesis of vitamins, the metabolism of xenobiotics, the renewal of gut epithelial cells, the participation in the development and activity of the immune system and functions in behavior (Turnbaugh et al., 2007).

Growing attention has been recently paid to the evidence supporting a role played by the gut microbiota in the pathogenesis of severe mental disorders in humans (Nguyen et al., 2018; Petra et al., 2015; Nemani et al., 2015; Dinan and Cryan, 2015). Through endocrinologic, neurologic, metabolic and immunologic pathways the microbiota and brain communicate bidirectionally (Dinan and Cryan, 2015; Anglin et al., 2015; Fond et al., 2016; Flowers and Ellingrod, 2015), forming the microbiota-gut-brain axis (Cryan and Dinan, 2012). A better understanding of the microbiota-gut-brain axis may lead to the development of novel biomarkers (Mancabelli et al., 2017), or treatment options that target the gut microbiota through modulation or supplementation or pharmacologically with psychobiotics (Dinan et al., 2013).

The microbiota communicates with the brain in several ways: it can secrete substances to the blood stream, it can induce immune cells to produce cytokines or it can alter signal transmission in the enteric or in the autonomic nervous system. Bacteria affect the production of several neurotransmitters, such as tryptophan, serotonin and kyneurenin (Anglin et al., 2015). Furthermore, the bacteria themselves can produce neurotransmitters such as norepinephrine, dopamine, serotonin, GABA, acetyl-choline or histamine (Flowers and Ellingrod, 2015). The brain, in its turn, influences the microbiota through the hypothalamic-pituitary-adrenal axis and the secretion of cortisol as well as sympathetic transmission, vagal transmission and their consequent changes in intestinal motility through gastroenteric secretions and changes in the production of mucin (Anglin et al., 2015). The study of the microbiota-gut-brain axis can have several potential translational applications ranging from its use as a biomarker (Mancabelli et al., 2017) to modulating it pharmacologically with the use of psychobiotics (Dinan et al., 2013).

The effects of the gut microbiota on behavior and cognition have been demonstrated in studies using germ-free animals which documented altered functioning of the hypothalamic-pituitary-adrenal axis and deficits in working memory tasks, as well as in studies using agents targeting the microbiota, such as probiotics, antibiotics or studies of infections (Cryan and Dinan, 2012). Most current studies in humans are observational case-control studies that have been recently reviewed (Järbrink-Sehgal and Andreasson, 2020). For major depressive disorder, a systematic review and meta-analysis additionally concluded that several common taxa were reduced in the patients' gut microbiota and the six included probiotic interventions reduced depressive symptoms (Sanada et al., 2020).

Differences in the microbiota have been documented in studies of stress and anxiety and of autism spectrum disorders (Sherwin et al., 2016). In addition to the comparative case-control studies, some other groups have focused on studying the effect of antipsychotic drugs (Hu et al., 2019; Flowers et al., 2019; Flowers et al., 2017; Pełka-Wysiecka et al., 2019; Gorbovskaya et al., 2019) or of electroconvulsive therapy (Kanayama et al., 2019) on the gut microbiota while other groups have explored the applicability of measuring the abundance of microbial taxa as a biomarker for bipolar disorder (Hu et al., 2019; Shen et al., 2018; Zheng et al., 2019) with promising results. Finally, the use of probiotics as treatment has been explored in clinical trials also with promising results in bipolar disorder and schizophrenia (Okubo et al., 2019; Aizawa et al., 2019; Reininghaus et al., 2018; Nagamine et al., 2018).

The recent studies that address this question specifically in bipolar disorder and schizophrenia have been reviewed elsewhere in early 2018 Nguyen et al., 2018 and in early 2019 T.T. Nguyen et al., 2019. At the time when the first review was conducted there were 10 studies about either the microbiota or microbial translocation in these two types of disorders. In this first review the authors additionally included five studies about bacterial translocation. In the second review they did not include studies about bacterial translocation and included 16 studies in total. Two studies looked at the oropharyngeal microbiome of schizophrenia. Eight studies found compositional and diversity differences in bipolar disorder. Two studies found differences in cases of first episode psychosis. In addition, there are two studies that have focused on schizophrenia and have also found significant differences. Finally, two studies have looked at the microbiota of bipolar disorder in a population of twins. One used only one pair of twins (Jiang et al., 2019) while Vinberg et all studied 128 monozygotic twins and found that high-risk twins had lower alpha diversity and absence of *Christencenellaceae* (Vinberg et al., 2019).

While previous studies show differences in the gut microbiota between the affected and non-affected individuals, the implicated taxa and their directionality largely vary (T.T. Nguyen et al., 2019). This can be due to differences between the clinical phenotyping or methods used for microbiota analysis, or failure to consider co-variates such as diet, medication, or smoking, thus potentially confounding the analysis. The present study selected gender and age matched cases and controls among siblings to account for genetic, lifestyle and environmental variation, and conducted thorough clinical and dietary assessments in parallel to the gut microbiota analysis. Our aim is to ask whether the gut microbiota of individuals affected with severe mental disorders is different from that of their healthy siblings.

2. Materials, subjects, and methods

2.1. Study subjects and design

2.1.1. Ethics statement

This study was approved by the Ethics Committee of the University of Costa Rica. All participants provided informed consent and signed a written informed consent form prior to their participation.

2.1.2. Participants

The Psychiatric Genetics research group of the Cellular and Molecular Biology Research Centre of the University of Costa Rica has recruited thousands of individuals for genetic studies over the years. These individuals have been evaluated for an accurate psychiatric diagnosis and several samples are stored from them (p.e. serum samples or induced pluripotent stem cell lines). From these previously recruited individuals, we recruited 37 subjects for the present study. They were all adults

living in the Central Valley of Costa Rica in the Greater Metropolitan Area of San José and, as per previous studies (Contreras et al., 2008; Pacheco et al., 2010) either diagnosed with a mental disorder or first-degree family members of someone with a mental disorder. In the original study, individuals were excluded if they were younger than 18 years or if they had either a learning disability or dementia. For this study, the 37 individuals were visited in their homes and asked to answer a questionnaire and provide a fecal sample. For the analysis, a subset of 26 participants, the "paired" subset, was selected by strict matching between affected and unaffected siblings. When there were several possible siblings that could be matched, we used criteria of gender and age for the final pairing.

2.2. Best estimate diagnosis

In the previous studies in which they had participated, the research subjects had undergone a Best Estimate Diagnosis (BED) procedure, this procedure has been described elsewhere (Contreras et al., 2009). Trained psychiatrists blind to previous diagnoses applied the Diagnostic Interview for Genetic Studies (DIGS) (Nurnberger et al., 1994) to each subject and in parallel trained psychologists applied the Family Interview for Genetic Studies (FIGS) (Diaz De Villalvilla Ramon et al., 2008) to a member of the family of the given subject. Afterwards, two other independent psychiatrists analyzed the information from both interviews and, when relevant, from medical records and then, together, reached a consensus diagnosis. The diagnoses were based on the DSM-IV diagnostic criteria.

2.3. Clinical assessment

The individuals answered a questionnaire designed by the American Gut Project (McDonald et al., 2018) about their habits and diet. Furthermore, they answered the Saltin Grimby scale for Physical Activity Level (Grimby et al., 2015), the Connor-Davidson (Connor and Davidson, 2003) scale for resilience, the WHO Disability Schedule 2.0 Disability and Schedule, 2017 (WHODAS 2.0), they underwent a physical examination of their musculoskeletal system and they answered the Schedule for Affective Disorders and Schizophrenia (SADS-C) (Endicott and Spitzer, 1978). Their weight, height and waist circumference were also measured. The body mass index (BMI) was calculated by dividing the weight in kilograms by the square of the height in meters. They were also asked about all regular medication as well as their monthly income.

2.4. Fecal sample collection and DNA purification from feces

Fresh fecal samples were collected, transported in ice and frozen as fast as possible and stored at −80 °C until analysis. The DNA was extracted using the 12,888–100 DNeasy PowerSoil Kit by QIAGEN (QIAGEN 2019). The purified DNA was subjected to concentration measurement by a Qubit® fluorometer (Invitrogen, CA 92,008, USA) and stored at −20 °C until further use.

2.5. Sequencing and preprocessing of the sequences

Illumina MiSeq sequencing of hypervariable V3-V4 regions of the 16S rRNA gene was performed according to the manual for Illumina with a slight modification where dual index Truseq-tailed 1-step amplification (Raju et al., 2018) was used for preparation. The pooled libraries were sequenced with an Illumina MiSeq instrument using MiSeq V3 reagent kit (2 × 300 bp) with 5% PhiX as spike-in at the sequencing unit of the Institute for Molecular Medicine Finland (FIMM), Helsinki, Finland. The forward reads were truncated to length of 150 bases with "ProcessReads" command or R package mare (Korpela, 2016), where the default settings for minimum quality score (2) and maximum expected errors (1) were applied. Reads with prevalence below 0.01% were removed. Truncated, filtered and dereplicated reads were annotated using the Silva database (Yilmaz et al., 2014). The median high-quality sequence per sample after preprocessing was 7097 (range 2331–9398).

2.5.1. Study groups

The selected 13 pairs of siblings were first divided into two groups: affected (A) or unaffected (U) according to the presence or absence of an axis I psychiatric disorder. The subjects were further divided into three groups: unaffected (UA), affected without psychosis (AA) and affected with psychosis (AP). The diagnoses of bipolar disorder type II, bipolar disorder type I without psychosis and major depressive episode without psychosis, were classified as "affected without psychosis" and those of bipolar disorder type I with psychosis, bipolar disorder non-otherwise specified, major depressive episode with psychosis, psychosis non-otherwise specified and schizoaffective disorder were classified as "affected with psychosis".

Gut microbiota analyses

All analyses were performed without rarefaction or data transformations. To account for the varying sequencing depth, the number of reads per sample was used as an offset in all statistical models. P-values generated from multiple comparison were adjusted using the Benjamini-Hochberg method for false discovery rates (FDR). FDR-adjusted p-values < 0.1 were considered significant and reported in the text. All analyses were performed in R Team, 2018 using RStudio (RStudio Team, 2020).

2.5.2. Identification of confounding variables

Permutational multivariate analysis of variance (PERMANOVA; *adonis* function in the *vegan* package (Oksanen et al., 2019)) based on the Bray-Curtis distance was used to identify factors that explain the variation in the microbiota, using all available background variables from the questionnaires. The R function "*GroupTest*" in the package mare (Korpela, 2016), which utilizes a negative binomial generalized linear model, was further used for differential abundance testing of individual bacterial taxa. The factors were subsequently considered as potential confounders if the same bacterial taxa appeared to be influenced by both the factors and mental disorders.

2.5.3. Comparison of the gut microbiota composition, richness, and diversity

The paired siblings were grouped in two (A and U, both $N = 13$) or three categories (UA; $N = 13$, AA $N = 4$ and AP; $N = 9$). When grouping in three categories, both AA and AP were compared to UA. The *GroupTest* function in *mare* was used to test differential abundances of the bacterial genera and families between the study groups adjusting for the selected confounders. The differences in alpha diversity and richness were tested using an analysis of variance (ANOVA).

2.6. Imputed metagenomic analysis

Bacterial metagenome content was predicted from 16S rRNA gene-based microbial compositions. Functional inferences were made from the Kyoto Encyclopedia of Gene and Genomes (KEGG) catalog (Kanehisa et al., 2012) using PICRUSt2 (Douglas et al., 2019). The "*DESeq*" function in DESeq2 (Love et al., 2014) was used to test for differentially abundant KEGG pathways between study groups adjusting for the use of levothyroxine and irbesartan as confounders.

3. Results

3.1. Basic characteristics of study participants

We recruited 37 individuals, all of Hispanic ethnicity and residing in the Greater Metropolitan Area of San José, Costa Rica. Initially they

Table 1
Psychiatric diagnoses of the participants after matching siblings.

Diagnosis	Number of participants	Presence of psychosis
No axis I diagnosis	13	No
Major Depressive Episode with psychotic features	1	Yes
Schizoaffective Disorder – Bipolar Type	2	Yes
Bipolar Affective Disorder, Type I with psychotic features	5	Yes
Bipolar Affective Disorder, Type I (without psychotic features)	3	No
Bipolar Affective Disorder – non otherwise specified	1	Yes
Bipolar Affective Disorder, Type II	1	No

comprised 22 individuals affected of a mental disorder and 15 unaffected controls who were first degree relatives of the affected participants. We then matched the participants to have one affected sibling paired with one healthy control sibling. This matching left us with 13 pairs of 26 siblings whose characteristics are summarized in Table 1 of the supplement. Of these, 8 individuals (four pairs) lived in the same household and 10 individuals (5 pairs) were neighbors. Their median age was of 56.5 years with a minimum of 37 and a maximum of 72 years. A total of 18 participants (69%) were women and 21 (80.8%) were not engaged in gainful activity or were responsible for domestic chores. Participants had different educational backgrounds, 15 of them had 6 or less years of formal education, 6 had 7–12 years and 5 had higher education. Only one affected individual smoked daily, the rest did not smoke. The mean BMI was of 29.9 with a minimum of 22 and a maximum of 42. The median income per capita was of 235 United States Dollars (USD) per month with a minimum of 13 and a maximum of 1176. Their diagnoses are summarized in Table 1. There were several co-morbidities: 7 individuals had diabetes mellitus type II, 11 suffered from hypothyroidism of which one was thyroidectomized for history of Hashimoto's thyroiditis, one unaffected sibling had Wilson's disease (Supplementary Table 2). The use of prescription drugs is listed in Supplementary Table 3.

Beta diversity assessment of the gut microbiota

To screen for potential confounders, we used PERMANOVA for beta diversity (i.e. between-samples distance) comparisons using all the available background variables among the whole data set of 37 individuals. We found that three variables were significantly associated to beta-diversity: use of levothyroxine (R2 = 0.065, p = 0.005), use of irbesartan (R2=0.068, p = 0.001) and birth by cesarean section (R2 = 0.057, p = 0.017). All other variables tested, including age, anthropometric measurements, use of psychoactive drugs (including antipsychotics), use of metformin, family identification number and dietary variables, were not significantly associated with the microbiota variation ($p > 0.05$). Based on the results, statistical analyses were adjusted for the use of levothyroxine and irbesartan. C-section birth was not used as confounder as only four individuals (two affected and two unaffected) were born by cesarean section and genus-level analysis indicated there was no overlap between the implicated taxa and those affected by the mental disorder status. The mental disorder status was not significantly associated to beta diversity (p = 0.809 and 0.743 for 2 and 3 group comparisons respectively) and neither was the family ID (p = 0.735).

3.2. Analysis of compositional differences in the gut microbiota

Among the 26 individuals, Firmicutes dominated the subjects' microbiota (60%), followed by Bacteroidetes (28%), Verrucobacteria (22%), Actinobacteria (8%), Proteobacteria (5%) and the rest represented less than 1%. At the genus level, the ten most abundant genera were *Lachnospiraceae IncertaeSedis* (14.6%), *Prevotella* (10.1%), *Bacteroides* (10.0%), *Faecalibacterium* (4.8%), *Blautia* (4.5%), *Ruminococcus* 4.2%), uncultured *Christensenellaceae* (3.1%), *Enterobater* (2.9%), *Megasphaera* (2.6%), and *Collinsella* (2.5%). Fig. 1 shows a composite plot for the abundant taxa stratified into three groups (Fig. 1).

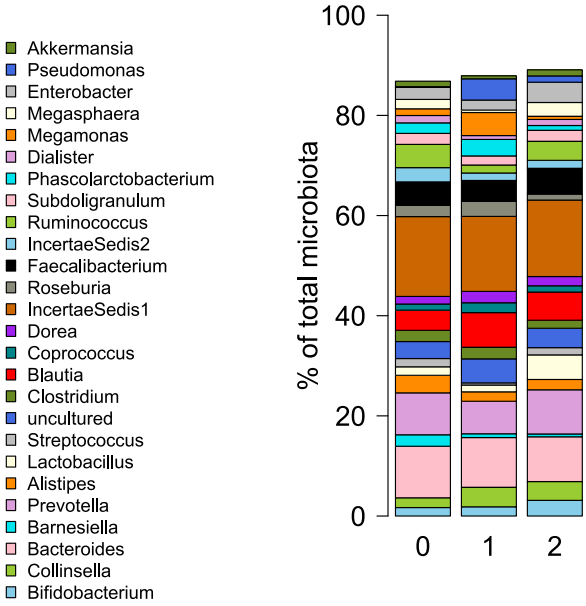

Fig. 1. Composition of the microbiota, shown as mean abundance of abundant genera, of the participants by groups unaffected (0), affected without psychosis (1) and affected with psychosis (2).

3.2.1. Siblings classified into affected or unaffected

The proportions of *Peptostreptococcaceae* (p = 0.086, fold change=0.27) and *Pseudomonadaceae* (p = 0.086, fold change=18.62) were significantly decreased and increased, respectively, in the affected individuals (Supplementary Table 4). At the genus level, the relative abundances of several bacterial taxa were reduced in the affected individuals, *Barnesiella* ($p = 4.11 \times 10^{-6}$, fold change=0.15), *IncertaeSedis Peptostreptoccocaceae* (p = 0.053, fold change=0.29), *Ruminococcaceae IncertaeSedis* (p = 0.080, fold change=0.43), *Acidaminococcus* ($p = 7.59 \times 10^{-10}$, fold change=0.42). *Megasphaera* ($p = 4.28 \times 10^{-67}$, fold change=1.82) and *Pseudomonas* (p = 0.076, fold change=18.62) were significantly enriched in the affected individuals (Fig. 2).

3.2.2. Siblings classified into unaffected, affected without psychosis and affected with psychosis

Several significant differences in the relative abundance of bacterial families and genera were observed when comparing the microbiota of the two affected study groups -affected without psychosis and affected with psychosis- to unaffected (Supplementary Table 5), including *Porphyromonadaceae* (reduced AP p = 0.017, fold change = 0.31), *Streptococcaaceaetococcaaceae* (AA p = 0.054, FC = 0.13), *Clostridiaceae* (AP p = 0.019, FC = 0.019), *Peptostreptococcaceae* (AP p = 0.052, FC = 0.5) and *Pasterurellaceae* (AA p = 0.054, FC=0.035) at the family level. At the genus level, as shown in Fig. 3, these differences were significant for *Barnesiella* (AA p = 0.078, AP $p = 6.72 \times 10^{-7}$), *Odoribacter* (AP p = 0.018; FC = 0.15) and *Paludibacter* (AA p = 0.078, FC = 26.05), *Lactococcus* (AP p = 0.025, FC = 5.72), *Clostridium* (AP p = 0.021, FC = 0.25), *Peptostrep-*

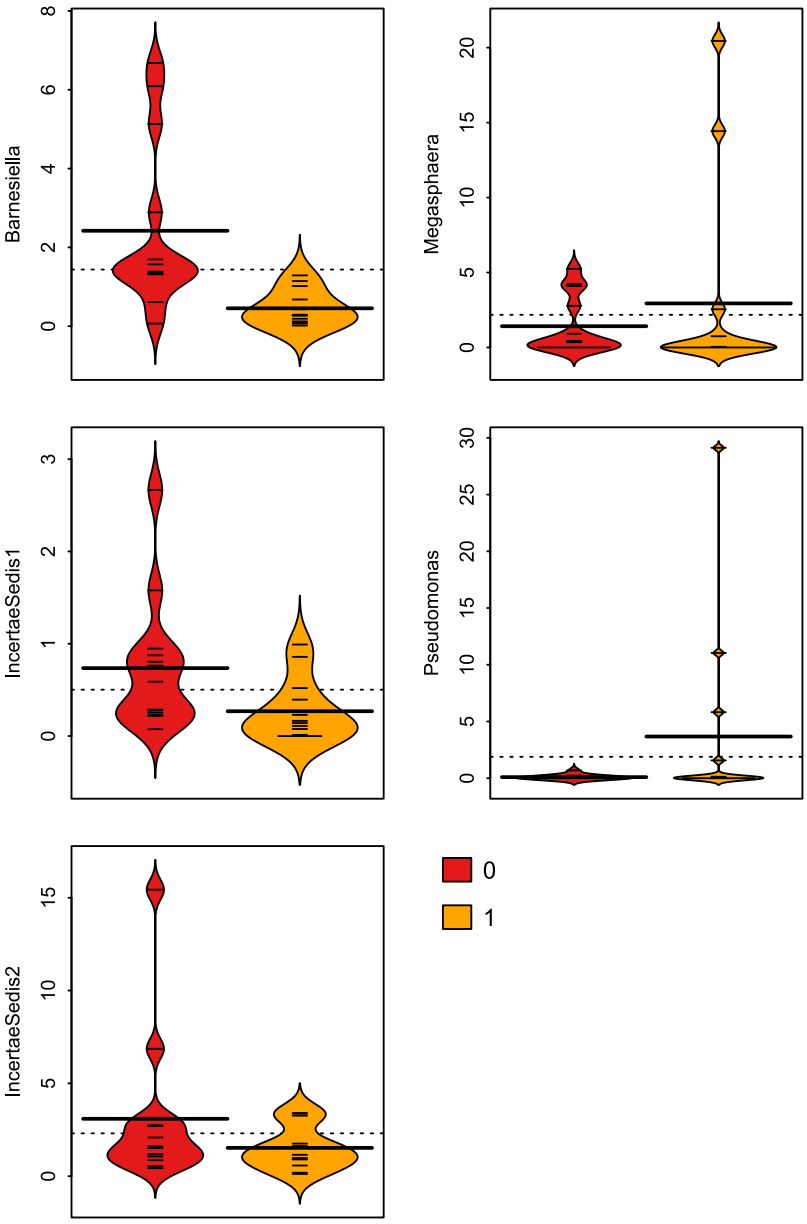

Fig. 2. Compositional differences that reached statistical significance at the genus level between unaffected (0) and affected (1) siblings. The *y*-axis refers to percentage of total microbiota.

tococcaceae IncertaeSedis (AP $p = 0.025$, FC=0.24), *Ruminococcaceae* IncertaeSedis (AP $p = 0.036$, FC = 0.37), *Acidaminococcus* (AP $p = 0.0028$, FC = 0.60), *Haemophilus* (AA $p = 0.078$, FC = 0.035).

3.3. Analysis of differences in diversity and richness

There was no significant difference in microbiota diversity when grouping by affected vs. unaffected ($p = 0.19$) or when grouping into unaffected, affected without psychosis and affected with psychosis ($p = 0.37$). When grouping the individuals into two groups (U and A), we found that richness was decreased by trend ($p = 0.08$) among affected participants but the finding did not reach significance. The difference was not significant when grouping the paired siblings into the three groups of unaffected, affected without psychosis and affected with pscyhosis ($p = 0.22$).

3.4. Analysis of differences in the predicted functions of the gut microbiota

Significant differences in the predicted functional potential of the gut microbiota between different study groups were identified ($p < 0.05$), (Figs. 4–6). Several pathways related to amino acid and fatty acid metabolism were significantly enriched in affected individuals (Fig. 4). The 3-group comparison revealed that the pathways related to amino acid metabolism, especially tryptophan biosynthesis, were significantly elevated in individuals with psychosis (Fig. 6). No difference in the predicted functions of the gut microbiota was observed between unaffected individuals and individuals affected without psychosis.

4. Discussion

We compared the gut microbiota of individuals with serious mental disorders to their healthy siblings and found significant differences in the abundance of bacterial taxa and predicted functions of the microbiota. To our knowledge, this is the first comparative study on the gut microbiota in individuals with mental disorders in Latin American populations, and one of the first to use sibling cohort for comparative microbiota study between cases and controls in any patient group. In addition to inbuild adjustment for genetic relatedness, common life history and largely shared diet and other environmental exposures, we additionally took extra effort to identify co-variates for the microbiota that

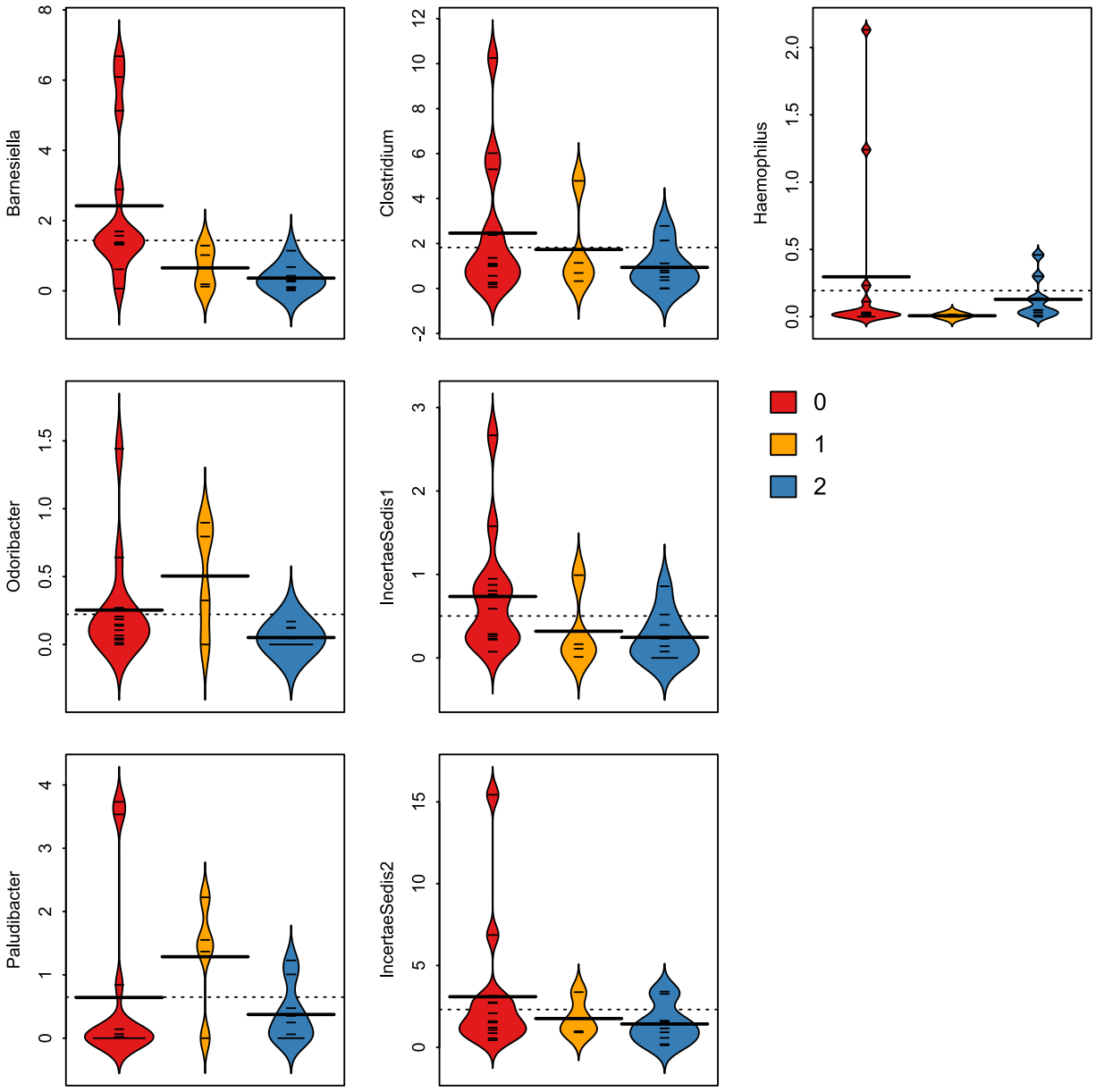

Fig. 3. Compositional differences that reached statistical significance at the genus level affected without psychosis (1) and affected with psychosis (2) compared to the unaffected group (0).

might confound the analyses. Somewhat surprisingly we found that two drugs, levothyroxine, used to treat hypothyroidism, and irbesartan, an angiotensin receptor II antagonist that is used as an antihypertensive, were strongly associated to microbiota variation, necessitating their use as confounders in the analyses. To our knowledge this is the first time levothyroxine or angiotensin II receptor antagonists have been reported to have an impact on the makeup of the gut microbiota. Interestingly, a previous study identifies levothyroxine use as a risk factor for small intestinal bacterial overgrowth (Brechmann et al., 2017). Our findings call for further studies on this topic and in general stress the importance of including all regular medication as a covariate in gut microbiota studies. Previously, drugs like metformin (Maier et al., 2018) or antipsychotics (Davey et al., 2012; Bahr et al., 2015) have been shown to influence the gut microbiota composition, which we did not observe in the present study.

Formal psychiatric diagnoses, as described in the DSM, are phenomenological and have little correlation with the biological pathophysiology of the disorder, providing a rationale for studying clusters of symptoms by grouping together disorders that share genes and symptoms rather than focusing on the specific diagnoses (Tamminga et al., 2013). In our small cohort of siblings, we therefore grouped several major psychiatric disorders into categories such as “affected”, “affected with psychosis” or “affected without psychosis”. This approach identified several differences in the gut microbiota between the affected and unaffected siblings (Figs. 2–6, Tables 4–5) that are discussed below.

Overall, our results indicate that most of the implicated taxa had lower relative abundance in affected versus unaffected individuals (Tables 4–5). This is in line with the recent systematic review and meta-analysis on the gut microbiota in major depressive disorder, (Sanada et al., 2020) however the taxa that the review identified as altered in at least two independent studies (e.g. *Faecalibacterium, Prevotella*) were different from the ones identified as altered in the current study on Latin Americans. Similarly to the above-mentioned meta-analysis, we did not find significant decrease in the richness or diversity of the microbiota in affected individuals although in both studies there was a trend for decreased richness compared to healthy controls.

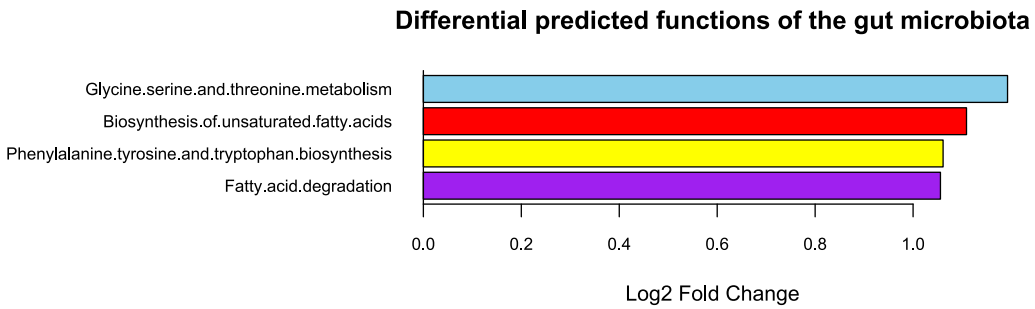

Fig. 4. Differences in the functional potential of the microbiota between U and A.

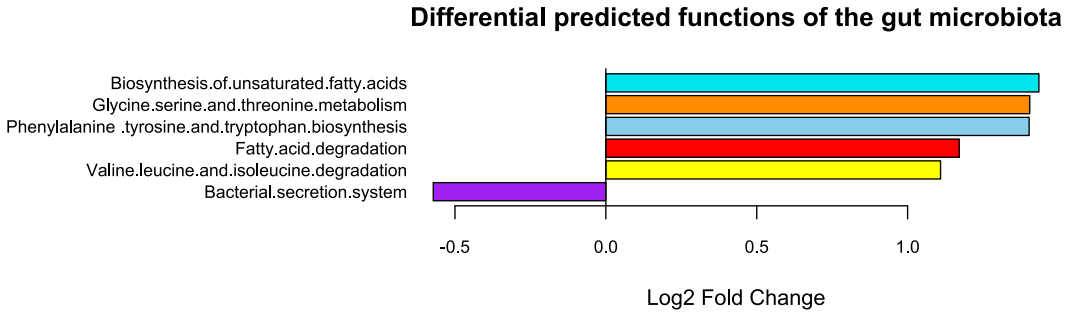

Fig. 5. Differences in functional potential of the gut microbiota between AP and UA.

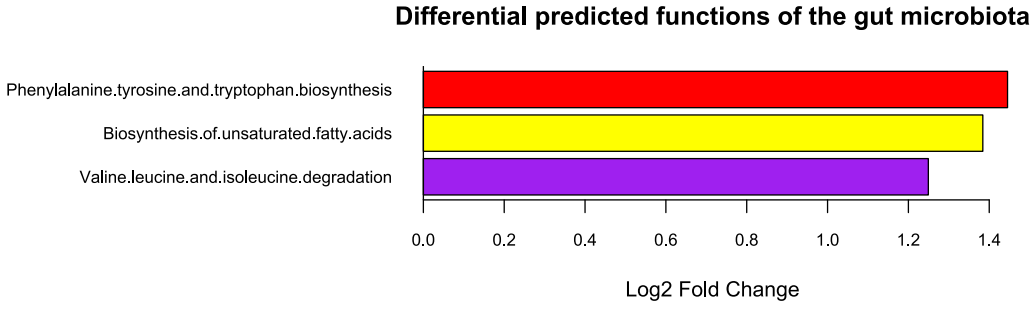

Fig. 6. Differences in functional potential of the gut microbiota between AP and AA.

In our cohort the largest difference in terms of fold change, almost 20-fold increase, was observed for genus *Pseudomonas. Pseudomonas* are generally considered to be pathogenic bacteria and were previously reported to be elevated in patients with depression (Huang et al., 2018) and schizophrenia (Maes et al., 2018). As gram-negative bacteria, *Pseudomonas* contain lipopolysaccharide (LPS) on their cell wall, which is a pro-inflammatory molecule (Pier, 2007). Our finding on increased *Pseudomonas* in affected individuals is therefore largely in accordance with the previous studies mentioned.

Ruminococcaceae and *Lachnospiraceae* are the two most dominant bacterial families in the human gut microbiota. *Ruminococcaceae* contains several butyrate producers and are hence generally considered as beneficial bacteria. A previous study found decreased in bipolar patients during depressive episodes (Painold et al., 2019). *Clostridiaceae* have been described as bacteria that participate in maintaining a healthy gut barrier and gut-associated immune system (Velasquez-Manoff, 2015). Our finding of a decrease in *Clostridiaceae* in affected individuals with psychosis is in line with these previous observations (Sanada et al., 2020).

Veillonellaceae (mainly *Megasphaera*) was enriched in the abundance in affected siblings compared to the unaffected. *Megasphaera* has been described to have anti-inflammatory activity and be neuroprotective (Ahmed et al., 2019). A few studies suggest that *Megasphaera* is associated with mental health, yet the mechanism remains unknown (Ahmed et al., 2019). *Porphyromonadaceae* have been found to be negatively correlated with cognition in elderly humans (Bajaj et al., 2016). In the present study we found significant decreases in the relative abundance of several genera within *Porphyromonadaceae*, including *Barnesiella, Odoribacter* and *Paludibacter*. The role of *Barnesiella* (Sakamoto et al., 2007) in the human gut ecosystem is poorly known, but it is generally associated with healthy outcomes (Ubeda et al., 2013; Daillère et al., 2016) and was reported as depleted in patients with depression. Similar fecal microbiota signatures have been found in patients with diarrhea-predominant irritable bowel syndrome and patients with depression (Liu et al., 2016). Comparably, the role of *Odoribacter* in the gut microbiota remains largely unclear, while it is thought to promote healthy barrier function of the gut (González-sarrías et al., 2018). *Odoribacter* was found more abundant in a transgenic mouse model of Alzheimer's Disease compared to a wild type strain of mice (Shen et al., 2016). It is of particular interest that *Barnesiella* was found dose-dependently decreased according to the severity of the state of being affected (Table 5), individuals without psychosis had the highest and those affected with psychosis the lowest levels of *Barnesiella*.

Our results show that there is a significant decrease in the abundance of oxygen-tolerant *Streptococcaceae* in affected compared to unaffected which contradicts previous studies in depressed individuals (Nguyen et al., 2018; Lin et al., 2017). On the other hand, the relative abundance of *Lactoccocus* was increased in individuals affected with

psychosis compared to unaffected siblings. Finally, among the *Pasteurellaceae, Haemophilus* has previously been documented to be decreased in schizophrenia (Nguyen et al., 2019) which is in line with our results.

The intestinal bacteria are highly individualized on compositional level, providing evolutionarily advantageous functional redundancy (Heintz-Buschart and Wilmes, 2018). Therefore, knowing the taxonomic composition provides little information regarding the functions involved in microbiota-host interaction. Few previous studies have investigated functional profiles of the gut microbiota between affected and unaffected individuals (Shen et al., 2018; Nguyen et al., 2019). Here we used a computational approach that utilizes 16S rRNA data to predict the functional composition of a metagenome. In line with the existing studies (Pełka-Wysiecka et al., 2019; Shen et al., 2018), several pathways related to amino acid metabolism were significantly elevated in individuals affected with mental disorders, especially in individuals affected with psychosis. Changes in glycine, serine and threonine metabolism have been reported to be associated with treatment-resistant depression (Maes et al., 1998). The branched-chain amino acids (BCAAs) valine, leucine and isoleucine have been proposed to be novel biomarkers for depression (Baranyi et al., 2016) and were increased peripherally in patients with schizophrenia in some studies (De Luca et al., 2008). Changes in tryptophan metabolism, which can be modulated by the gut microbiota (O'Mahony et al., 2015), are believed to be involved in the pathogenesis of psychiatric disorders (Gostner et al., 2020). In summary, our findings suggest that altered amino acid metabolism in the gut microbiota is associated with serious mental disorders.

The main strengths of this study are the carefully screened participants and their matching siblings, which reduces potential noise arising from genetic or environmental factors. Imputed metagenomics that complements our taxonomic analysis provides additional information with regards to the specific metabolic pathways potentially involved in host-microbiota interactions relevant for mental health.

Limitations of our study include small sample size and the cross-sectional design. Indeed, the small size of the sample limits its statistical power and makes it more vulnerable to confounding factors. There is also the matter of the limited uniformity of the sample. Although numerous confounding factors were utilized in the PERMANOVA, a more uniform sample would have been desirable. Finally, the cross-sectional design of the study does not allow for the establishment of causality and its directionality. Future longitudinal studies with larger cohorts are warranted to confirm the findings from our study, such as changes in amino acid metabolism of the gut microbiota in individuals affected with mental disorders, which may represent novel therapeutic options.

In conclusion, our results recapitulate several observations made by previous studies, such as increased LPS-producing *Pseudomonas* and reduced proportions of butyrate producing *Ruminococcaceae* in affected individuals. We also identified novel associations between serious mental disorders and the low-abundant genera *Barnesiella* and *Odoribacter*. The changes in the taxonomic composition of the gut microbiota may contribute to altered amino acid metabolism, which has been implicated in several psychiatric disorders. Taken together, our study adds to the current evidence on the importance of the gut-brain axis in the pathophysiology of serious mental disorders.

Declaration of Competing Interest

All the authors declare that they have no conflict of interest.

Acknowledgments

The study was supported by University of Helsinki (3-year research grant and principal investigator grant within the Human Microbiome Research Program funding to Anne Salonen).

We would also like to thank Dr Javier Mora from the Helminths Laboratory of the Faculty of Microbiology of the University of Costa Rica for his team's help in the sample collection and extraction of DNA.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jadr.2021.100081.

References

Ahmed, S., et al., 2019. In vitro characterization of gut Microbiota-derived bacterial strains with neuroprotective properties. Front. *Cell. Neurosci.* 13, 1–17.
Aizawa, E., et al., 2019. Bifidobacterium and Lactobacillus counts in the gut microbiota of patients with bipolar disorder and healthy controls. Front. Psychiatry 10, 1–8.
Al-Diwani, A.A., Pollak, T.A., Irani, S.R., Lennox, B.R., 2017. Psychosis: an autoimmune disease? ARPN J. Eng. Appl. Sci. 152, 388–401.
American Psychiatric Association, 2013. *DSM-V. Am. J. Psychiatry* doi:10.1176/appi.books.9780890425596.744053.
Anglin, R., Surette, M., Moayyedi, P., Bercik, P., 2015. Lost in translation: the Gut Microbiota in psychiatric illness. Can. J. Psychiatry 60, 460–463.
Anttila, V., et al., 2018. Analysis of shared heritability in common disorders of the brain. Science 360 (80), eaap8757.
Arciniegas, D.B., 2015. Psychosis Continuum. 21, 715–736.
Bahr, S.M., et al., 2015. Use of the second-generation antipsychotic, risperidone, and secondary weight gain are associated with an altered gut microbiota in children. Transl. Psychiatry 5, e652.
Bajaj, J.S., et al., 2016. Elderly patients have an altered gut-brain axis regardless of the presence of cirrhosis. Sci. Rep. 6, 1–12.
Baranyi, A., et al., 2016. Branched-chain amino acids as new biomarkers of major depression - a novel neurobiology of mood disorder. PLoS One 11, 1–10.
Blekhman, R., et al., 2015. Host genetic variation impacts microbiome composition across human body sites. Genome Biol. 16, 191.
Brechmann, T., et al., 2017. Levothyroxine therapy and impaired clearance are the strongest contributors to small intestinal bacterial overgrowth: results of a retrospective cohort study. World J. Gastroenterol. 23, 842–852.
Collaborators, G.B., 2015. of D. S. 2013. Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 386, 743–800.
Connor, K.M., Davidson, J.R.T, 2003. Development of a new Resilience scale: the Connor–Davidson Resilience scale (CD-RISC). Depress. Anxiety 18, 76–82.
Contreras, J., Montero, P., Dassori, A., Escamilla, M., Raventós, H., 2008. Caracterización de un grupo de pacientes con esquizofrenia en el Valle Central de Costa Rica. Amc 50, 153–159.
Contreras, J., et al., 2009. Diagnosis of Schizophrenia in latino populations. J. Nerv. Ment. Dis. 197, 530–535.
Cryan, J.F., Dinan, T.G., 2012. Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. Nat. Rev. Neurosci. 13, 701–712.
Daillère, R., et al., 2016. Enterococcus hirae and Barnesiella intestinihominis facilitate cyclophosphamide-induced therapeutic immunomodulatory effects. Immunity 45, 931–943.
Davey, K.J., et al., 2012. Gender-dependent consequences of chronic olanzapine in the rat: Effects on body weight, inflammatory, metabolic and microbiota parameters. Psychopharmacology 221, 155–169.
De Luca, V., et al., 2008. Peripheral amino acid levels in schizophrenia and antipsychotic treatment. Psychiatry Investig 5, 203–208.
Diaz De Villalvilla Ramon, T., et al., 2008. Spanish version of the Family Interview for Genetic Studies (FIGS). Actas Esp. Psiquiatr. 36, 20–24.
Dinan, T.G., Cryan, J.F., 2015. The impact of gut microbiota on brain and behaviour: implications for psychiatry. Curr. Opin. Clin. Nutr. Metab. Care 18, 552–558.
Dinan, T.G., Stanton, C., Cryan, J.F., 2013. Psychobiotics: a novel class of psychotropic. Biol. Psychiatry 74, 720–726.
Disability, W.H.O. & Schedule, A. Disabil Rehabil. 2017 Nov;39(23):2347-2380. doi:10.1080/09638288.2016.1223177. Epub 2016 Nov 7. World Health Organization disability assessment schedule 2.0: An international systematic review Stefano Federici 1 , Marco Bracalenti 1 , Fabio Meloni 1 , Juan V Luciano 2 3 Affiliations PMID: 27820966 doi:10.1080/09638288.2016.1223177.
Douglas, G.M. et al. PICRUSt2: An improved and extensible approach for metagenome inference Gavin M. Douglas, Vincent J. Maffei, Jesse Zaneveld, Svetlana N. Yurgel, James R. Brown, Christopher M. Taylor, Curtis Huttenhower, Morgan G. I. Langille doi:10.1101/672295. *bioRxiv* (2019).
Endicott, J., Spitzer, R., 1978. The schedule for affective disorders and Schizophrenia. Arch. Gen. Psychiatry 35, 837–844.
Flowers, S.A., Ellingrod, V.L., 2015. The microbiome in mental health: potential contribution of gut microbiota in disease and pharmacotherapy management. Pharmacotherapy 35, 910–916.
Flowers, S., Evans, S., Ward, K., McInnis, M., Ellingrod, V., 2017. Interaction between atypical antipsychotics and the gut microbiome in a bipolar disease cohort. Pharmacotherapy 37, 261–267.
Flowers, S.A., et al., 2019. Effects of atypical antipsychotic treatment and resistant starch supplementation on gut microbiome composition in a cohort of patients with bipolar disorder or Schizophrenia. Pharmacotherapy 39, 161–170.
Fond, G., Chevalier, G., Eberl, G., Leboyer, M., 2016. Le rôle potentiel du microbiote intestinal dans les troubles psychiatriques majeurs : mécanismes, données fondamentales, comorbidités gastro-intestinales et options thérapeutiques. Press. Medicale 45, 7–19.
Girgis, R.R., Kumar, S.S., Brown, A.S., 2014. The cytokine model of schizophrenia: emerging therapeutic strategies. Biol. Psychiatry 75, 292–299.

González-sarrías, A., et al., 2018. The endotoxemia marker lipopolysaccharide-binding protein is reduced in overweight-obese subjects consuming pomegranate extract by modulating the gut microbiota : a randomized clinical trial. Mol. Nutr. Food Res. 1–32. doi:10.1002/mnfr.201800160.

Gorbovskaya, I., et al., 2019. Investigation of the gut microbiome in patients with schizophrenia and clozapine-induced weight gain: protocol and clinical characteristics of first patient cohorts. Neuropsychobiology doi:10.1159/000494696.

Gostner, J.M., et al., 2020. Tryptophan metabolism and related pathways in psychoneuroimmunology: the impact of nutrition and lifestyle. Neuropsychobiology 79, 89–99.

Grimby, G., et al., 2015. The 'Saltin-Grimby Physical Activity Level Scale' and its application to health research. Scand. *J. Med. Sci. Sport.* 25, 119–125.

Heintz-Buschart, A., Wilmes, P., 2018. Human gut microbiome: function matters. Trends Microbiol. 26, 563–574.

Hu, S., et al., 2019. Gut microbiota changes in patients with bipolar depression. Adv. Sci. doi:10.1002/advs.201900752.

Huang, Y., et al., 2018. Possible association of firmicutes in the gut microbiota of patients with major depressive disorder. Neuropsychiatr. Dis. Treat. 14, 3329–3337.

Järbrink-Sehgal, E., Andreasson, A., 2020. The gut microbiota and mental health in adults. Curr. Opin. Neurobiol. 62, 102–114.

yinlian Jiang, H., Xu, L., Zhang, X., Zhang, Z., Ruan, B., 2019. The microbiome in bipolar depression: a longitudinal study of one pair of monozygotic twins. Bipolar Disord. 21, 93–97.

Kanayama, M., Hayashida, M., Hashioka, S., Miyaoka, T., Inagaki, M., 2019. Decreased clostridium abundance after electroconvulsive therapy in the gut microbiota of a Patient with Schizophrenia. Case Rep. *Psychiatry* 2019, 57–60.

Kanehisa, M., Goto, S., Sato, Y., Furumichi, M., Tanabe, M., 2012. KEGG for integration and interpretation of large-scale molecular data sets. Nucl. Acids. Res. 40, 109–114.

Knight, R., et al., 2017. The microbiome and human biology. Annu. Rev. Genom. Hum. Genet. 18, 65–86.

Korpela, K. Microbiota analysis in R easily. R package version 1.0. *GitHub*https://github.com/katrikorpela/mare (2016).

Lin, P., et al., 2017. Prevotella and Klebsiella proportions in fecal microbial communities are potential characteristic parameters for patients with major depressive disorder. J. Affect. Disord. 207, 300–304.

Liu, Y., et al., 2016. Similar fecal microbiota signatures in patients with diarrhea-predominant irritable bowel syndrome and patients with depression. Clin. Gastroenterol. Hepatol. 14, 1602–1611 e5.

Love, M.I., Huber, W., Anders, S., 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 15, 1–21.

Maes, M., Vandoolaeghe, E., Scharpe, S., 1998. Excitatory amino acids in treatment-resistant depression. Acta Psychiatr. Scand. 97 (4), 302–308.

Maes, M., Kanachanatawan, B., Sirivichayakul, S., Carvalho, A.F., 2018. In Schizophrenia, increased plasma IgM / IgA responses to gut commensal bacteria are associated with negative symptoms, neurocognitive impairments, and the deficit phenotype. Neurotox. Res. doi:10.1007/s12640-018-9987-y.

Maier, L., et al., 2018. Extensive impact of non-antibiotic drugs on human gut bacteria Europe PMC Funders Group. Nature 555, 623–628.

Mancabelli, L., et al., 2017. Identification of universal gut microbial biomarkers of common human intestinal diseases by meta-analysis. FEMS Microbiol. Ecol. 93, 1–10.

McDonald, D., et al., 2018. American gut: an open platform for citizen-science microbiome research. bioRxiv 277970. doi:10.1101/277970.

Methé, B., 2012. A framework for human microbiome research. Nature 486, 215–221.

Nagamine, T., Ido, Y., Nakamura, M., Okamura, T., 2018. 4G-β-D-galactosylsucrose as a prebiotics may improve underweight in inpatients with schizophrenia. Biosci. Microbiota, Food Heal. 37, 45–47.

Nemani, K., Hosseini Ghomi, R., McCormick, B., Fan, X., 2015. Schizophrenia and the gut-brain axis. Prog. Neuropsychopharmacol. Biol. Psychiatry 56, 155–160.

Nguyen, T.T., Kosciolek, T., Eyler, L.T., Knight, R., Jeste, D.V., 2018. Overview and systematic review of studies of microbiome in schizophrenia and bipolar disorder. J. Psychiatr. Res. 99, 50–61.

Nguyen, T.T., Hathaway, H., Kosciolek, T., Knight, R., Jeste, D.V., 2019a. Gut microbiome in serious mental illnesses: a systematic review and critical evaluation. Schizophr. Res. doi:10.1016/j.schres.2019.08.026.

Nguyen, T.T., et al., 2019b. Differences in gut microbiome composition between persons with chronic schizophrenia and healthy comparison subjects. Schizophr. Res. 204, 23–29.

Nurnberger, J.I., et al., 1994. Diagnostic interview for genetic studies. Arch. Gen. Psychiatry 51, 849.

O'Mahony, S.M., Clarke, G., Borre, Y.E., Dinan, T.G., Cryan, J.F., 2015. Serotonin, tryptophan metabolism and the brain-gut-microbiome axis. Behav. Brain Res. 277, 32–48.

Oksanen, J., et al., 2019. Package 'vegan' title community ecology package. Commun. Ecol. Packag..

Okubo, R., et al., 2019. Effect of bifidobacterium breve A-1 on anxiety and depressive symptoms in schizophrenia: a proof-of-concept study. J. Affect. Disord. 245, 377–385.

Pacheco, A., et al., 2010. Social and clinical comparison between schizophrenia and bipolar disorder type I with psychosis in Costa Rica. Soc. Psychiatry Psychiatr. Epidemiol. 45, 675–680.

Painold, A., et al., 2019. A step ahead: exploring the gut microbiota in inpatients with bipolar disorder during a depressive episode. Bipolar Disord. 21, 40–49.

Pełka-Wysiecka, et al., 2019. Analysis of gut microbiota and their metabolic potential in patients with schizophrenia treated with olanzapine: results from a six-week observational prospective cohort study. J. Clin. Med. 8, 1605.

Petra, A.I., et al., 2015. Gut-microbiota-brain axis and its effect on neuropsychiatric disorders with suspected immune dysregulation. Clin. Ther. 37.

Pier, G.B., 2007. Pseudomonas aeruginosa lipopolysaccharide: a major virulence factor, initiator of inflammation and target for effective immunity. Int. J. Med. Microbiol. 297, 277–295.

QIAGEN. DNeasy PowerSoil Kit. https://www.qiagen.com/fi/products/dneasy-powersoil-kit/#resources (2019).

Raju, S.C., et al., 2018. Reproducibility and repeatability of six high-throughput 16S rDNA sequencing protocols for microbiota profiling. J. Microbiol. Methods 147, 76–86.

Reininghaus, E.Z., et al., 2018. Probiotic treatment in individuals with euthymic bipolar disorder: a pilot-study on clinical changes and compliance. Neuropsychobiology doi:10.1159/000493867.

RStudio Team (2020). RStudio: Integrated Development for R. RStudio, PBC, Boston, MA URL http://www.rstudio.com/.

Team, R.C. R: a Language and Environment for Statistical Computing. (2018).

Sakamoto, M., Lan, P.T.N., Benno, Y, 2007. Barnesiella viscericola gen. nov., sp. nov., a novel member of the family Porphyromonadaceae isolated from chicken caecum. Int. J. Syst. Evol. Microbiol. 57, 342–346.

Sanada, K., et al., 2020. Gut microbiota and majore depressive disorder: a systematic review and meta-analysis. J. Affect. Disord. 266, 1–13.

Shen, L., Liu, L., Ji, H.-F., 2016. Alzheimer's disease histological and behavioral manifestations in transgenic mice correlate with specific gut microbiome state. J. Alzheimer's Dis. doi:10.3233/JAD-160884.

Shen, Y., et al., 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.

Sherwin, E., Rea, K., Dinan, T.G., Cryan, J.F., 2016. A gut (microbiome) feeling about the brain. Curr. Opin. Gastroenterol. 32, 96–102.

Sullivan, P.F., et al., 2018. Psychiatric genomics: an update and an Agenda. Am. J. Psychiatry 175, 15–27.

Tamminga, C.A., et al., 2013. Clinical Phenotypes of psychosis in the Bipolar-Schizophrenia Network on Intermediate Phenotypes (B-SNIP). Am. J. Psychiatry 170, 1263–1274.

Tamminga, C.A., et al., 2017. Strategies for advancing disease definition using biomarkers and genetics: the bipolar and schizophrenia network for intermediate phenotypes. Biol. Psychiatry Cogn. Neurosci. Neuroimaging 2, 20–27.

Turnbaugh, P.J., et al., 2007. The human microbiome project. Nature 449, 804–810.

Ubeda, C., et al., 2013. Intestinal microbiota containing Barnesiella species cures vancomycin-resistant Enterococcus faecium colonization. Infect. Immun. 81, 965–973.

Velasquez-Manoff, M., 2015. The peace - keepers. Nature 516.

Vinberg, M., et al., 2019. Remitted affective disorders and high familial risk of affective disorders associate with aberrant intestinal microbiota. Acta Psychiatr. Scand. 139, 174–184.

Yilmaz, P., et al., 2014. The SILVA and 'all-species Living Tree Project (LTP)' taxonomic frameworks. Nucl. Acids. Res. 42, 643–648.

Zheng, P., et al., 2019. The gut microbiome from patients with schizophrenia modulates the glutamate-glutamine-GABA cycle and schizophrenia-relevant behaviors in mice. Sci. Adv. 5, eaau8317.