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Gut Microbiota in Psychiatric Disorders: A Systematic Review

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ABSTRACT

Objective: This systematic review sought to comprehensively summarize gut microbiota research in psychiatric disorders following PRISMA guidelines.

Methods: Literature searches were performed on databases using keywords involving gut microbiota and psychiatric disorders. Articles in English with human participants up until February 13, 2020, were reviewed. Risk of bias was assessed using a modified Newcastle-Ottawa Scale for microbiota studies.

Results: Sixty-nine of 4231 identified studies met the inclusion criteria for extraction. In most studies, gut microbiota composition differed between individuals with psychiatric disorders and healthy controls; however, limited consistency was observed in the taxonomic profiles. At the genus level, the most replicated findings were higher abundance of *Bifidobacterium* and lower abundance of *Roseburia* and *Faecalibacterium* among patients with psychiatric disorders.

Conclusions: Gut bacteria that produce short-chain fatty acids, such as *Roseburia* and *Faecalibacterium*, could be less abundant in patients with psychiatric disorders, whereas commensal genera, for example, *Bifidobacterium*, might be more abundant compared with healthy controls. However, most included studies were hampered by methodological shortcomings including small sample size, unclear diagnostics, failure to address confounding factors, and inadequate bioinformatic processing, which might contribute to inconsistent results. Based on our findings, we provide recommendations to improve quality and comparability of future microbiota studies in psychiatry.

Key words: autism spectrum disorder, major depressive disorder, anorexia nervosa, bipolar disorder, schizophrenia, attention-deficit/hyperactivity disorder, gut microbiota.

INTRODUCTION

The gut microbiota has extensive reciprocal connections with the human brain through microbial metabolites, the vagus nerve and hormonal and immunological signaling, collectively forming the microbiome-gut-brain axis (1,2). Animal studies suggest a possible influence of gut microbiota on behavior. Using microbiota-depleted or axenic rodents, researchers were able to replicate pathognomonic behaviors in animals after transplantation with fecal microbiota from humans with depression (3,4), schizophrenia (SCZ) (5), or attention-deficit/hyperactivity disorder (ADHD) (6). Similarly, colonization of axenic mice with fecal microbiota from children with autism spectrum disorder (ASD) induced autism-like behavior (7). However, these studies are commonly limited by the small number of human donors, and findings from animal experiments are not easily transferable to humans (8).

Targeting the gut microbiota might provide new therapeutic avenues for psychiatric disorders, but whether and how the microbiota differs in individuals with psychiatric disorders is insufficiently understood (7). The number of studies investigating the differences in gut microbiota between healthy controls and patients with psychiatric disorders has accelerated with increased interest and affordability of high-throughput sequencing techniques (9). A number of systematic

Abbreviations: ADHD = attention-deficit/hyperactivity disorder, AN = anorexia nervosa, ANX = anxiety disorders, ASD = autism spectrum disorder, BMI = body mass index, BPD = bipolar disorder, DSM = Diagnostic and Statistical Manual for Psychiatric Disorders, ED = eating disorders, ICD-10 = International Classification of Disease, Tenth Revision, MDD = major depressive disorder, MNOS = modified Newcastle-Ottawa Scale, NGS = next-generation sequencing, SCFA = short-chain fatty acids, SCZ = schizophrenia

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Microbiota Terminology: **High-throughput sequencing** = a group of sequencing techniques that allow for large-scale genome sequencing by processing multiple DNA sequences in parallel, **Marker-gene analysis** = amplification of target genes to determine the presence and abundance of microbes. 16S rRNA is a highly conserved gene among bacteria and archaea commonly used as a marker gene in studies of microbiota. This technique uses universal primers targeting the hypervariable regions (V1–V9) of ribosomal RNA, **Metagenome analysis (shotgun)** = uses the entire genome of all microorganisms for whole-genome sequencing, which offers higher resolution compared with marker-gene analysis and allows for functional characterization of microbial communities, **Alpha diversity** = refers to the diversity within a sample. It can measure richness of species (observed species, Chao1, ACE index) or how evenly distributed the microbes are (Shannon and Simpson index), **Beta diversity** = refers to diversity between samples. Different metrics can be used to measure beta diversity. Bray-Curtis dissimilarity measures differences in microbial abundance between samples. Unifrac takes phylogenetics or relatedness of species into account, and it can be weighted to the relative abundance of species (weighted Unifrac)

reviews have sought to compile these findings; however, most focused on a limited number of related psychiatric disorders rather than a comprehensive overview of the field (10–17).

Although the field is relatively young and many studies use different experimental designs and methodology, we contend that there is a need for a comprehensive systematic review of microbiota studies, including all major categories of psychiatric disorders, at this stage of the science. We included all case-control studies that used high-throughput sequencing techniques for analysis, which, to our knowledge, has not been done before.

Our primary objective is to compare gut microbiota in patients with a psychiatric disorder (i.e., ADHD, anxiety disorders [ANX], ASD, bipolar disorder [BPD], eating disorders (EDs), major depressive disorder [MDD], tics, obsessive-compulsive disorders, posttraumatic stress disorder, SCZ) with healthy controls. Alpha diversity, average species diversity in a sample, and beta diversity, similarities in diversity between samples and taxonomic differences across the psychiatric disorders, are the primary outcome measures. Secondary outcome measures are differences in microbial metabolites and associations between taxonomic differences and clinical features.

METHODS

Protocol and Registration

This systematic review has been preregistered at PROSPERO under the identification number CRD42019132642. PRISMA guidelines for a systematic review or meta-analysis have been followed (18).

Eligibility Criteria

The primary objective was to compile evidence of differences in fecal microbiota as a proxy for microbiota of the lower gastrointestinal tract between patients with psychiatric disorders and healthy individuals. Therefore, only original observational studies using a case-control, cohort, or cross-sectional design with a healthy control group were included. We had no restriction regarding the age of participants. Animal studies, studies without a diagnosed psychiatric disorder, interventional studies, and studies that did not use high-throughput sequencing techniques were excluded.

Information Sources

Human studies in English up until February 13, 2020, were searched in Medline, Embase, Cochrane, and Web of Science and Psychinfo, with

support from Karolinska Institutet University Library, using the following search string (for Web of Science): ((anxiety or “obsessive-compulsive” or paranoi* or panic or phobi* or “anorexia nervosa” or “appetite disorder*” or “binge-eating” or bulimia or “compulsive eat*” or “eating disorder*” or “feeding disorder*” or attention-deficit/hyperactivity disorder or asperger* or “attention deficit” or autistic or autism or “bipolar disorder” or depression or “depressive disorder*” or mania* or manic or ptsd or “post-traumatic*” or schizophrenia or schizophrenic* or “stress disorder*” or tic or tics or tourette*)) AND (“enteric bacteria” or “gastr* flora” or “gut flora” or “intestinal flora” or microbiom* or microbiot* or microbial* or microflora* or mycobiome*) NOT (“animal*” or “guinea pig*” or “horse*” or “mice” or “mouse” or “rat” or “rats”)) (Supplementary Material 1, <http://links.lww.com/PSYMED/A743>). The gathered articles were deduplicated. Gray literature, such as dissertations, reports, and manuscripts, was included if inclusion criteria were fulfilled. Reference lists from other reviews were used for reference checking.

Study Selection and Data Collection Process

A single Endnote file was exported to Rayyan.com (19). Two reviewers, blinded to each other’s assessments, sorted articles based on title and abstract. Reasons for exclusion were standardized according to the terms: review, animal studies, gray literature that did not meet eligibility criteria, not relevant, case series/case reports, and not retrievable. Subsequently, full-text articles were assessed for eligibility. If the article did not meet the inclusion criteria, it was excluded according to the following terms: not a microbiota study, not a study of a psychiatric disorder, or irrelevant study design. If the reviewers had disparate opinions about inclusion, group consensus was achieved through discussion. A priori determined outcome variables were subdivided into four categories: general information about each study, study design and demographic variables, methodological information, and outcome variables (alpha and beta diversity, and taxonomic abundance of bacterial groups; microbial metabolites; and association with clinical features). No assumptions or simplifications were made during extraction. If the reported data from the study were absent or ambiguous, the cell was filled in as not available (NA). No deviations from the prespecified protocol occurred. All extracted information was double checked by another reviewer to ensure that it was correctly extracted.

Assessment of Risk of Bias

Two reviewers independently conducted quality assessments of all included studies using the Newcastle-Ottawa Scale (NOS) (20). The original NOS is a tool to assess the quality of nonrandomized studies in systematic reviews and meta-analyses. However, the exposure items are not applicable for most observational cross-sectional studies. We therefore modified the scale to more adequately represent the study quality by replacing exposure items with methodological items (Supplementary Material 2, <http://links.lww.com/PSYMED/A744>). Because this is a field that relies heavily on emerging technology, consistency of methodological reporting is critical to ensure reproducibility. Thus, the modified Newcastle-Ottawa Scale (MNOS) contains eight items, categorized into three topics: selection, comparability, and methodology. We chose age as the most important confounding factor to examine comparability across studies (21). Discrepancies in quality assessment were resolved by discussion among the reviewers.

Statistical Analysis

Spearman correlation was used for correlation analysis between study quality (MNOS score) and time of publication. R packages were used for statistical analysis and figures (22). The reported taxonomic differences between cases and healthy controls across psychiatric disorders were summarized as a heat-map to identify replicated patterns despite differences in hypervariable regions and bioinformatic processes. We only included studies that used 16S rRNA sequencing as the sequencing method. We chose to map genus-level

differences based on the resolution of the sequencing method (23). If a genus was not reported in more than one study, it was excluded. The following values were assigned to “increase,” “decrease,” and “no report/no change” in abundance, respectively: +1, -1, and 0. Next, the values were weighted by the sample size in a disorder-specific manner. Finally, the sum of weighted values of each genus in different studies per disorder was visualized in the heat-map, with disorders being listed in decreasing order according to number of studies included.

RESULTS

Study Selection

Of 4231 identified studies, 2682 articles were screened, and 341 articles went to full-text assessment for eligibility. Sixty-nine articles met the inclusion criteria and were included in this review (4,5,24–89) (Figure 1).

Study Characteristics

From 2015 forward, the number of studies in this field increased rapidly. A Spearman correlation test showed a weak positive correlation between overall study quality as measured with MNOS

and date of publication, which was not statistically significant ($r_s = 0.025$, $p = .84$; Figure 2). Although the difference in study quality was large between individual studies, the general study quality has not improved over time (Supplementary Table 1, <http://links.lww.com/PSYMED/A745>). Most studies were conducted in Europe, the United States, and, more recently, China, contributing approximately 30% each to the total number of publications (Supplementary Table 2, <http://links.lww.com/PSYMED/A746>).

Demographic characteristics of each study are presented in Supplementary Table 3, <http://links.lww.com/PSYMED/A747>. A total of 2880 cases, with a mean number of 42 cases per study, have been included in these 69 studies. The average number of controls for every case was less than 1:1. In fact, 37 of the articles had more cases than controls. Excluding ASD studies, the mean age was 34.5 years, ranging from 8.4 to 52.9 years. Because ASD studies mainly recruited children, the mean age was 6.5 years, ranging from 2 to 14.4 years. Notably, 47 of 69 studies did not use age-matched controls. The sex in ASD studies was heavily weighted toward boys (male-to-female ratio, >5:1), which could be due to earlier age of onset of ASD in boys. In contrast, ED

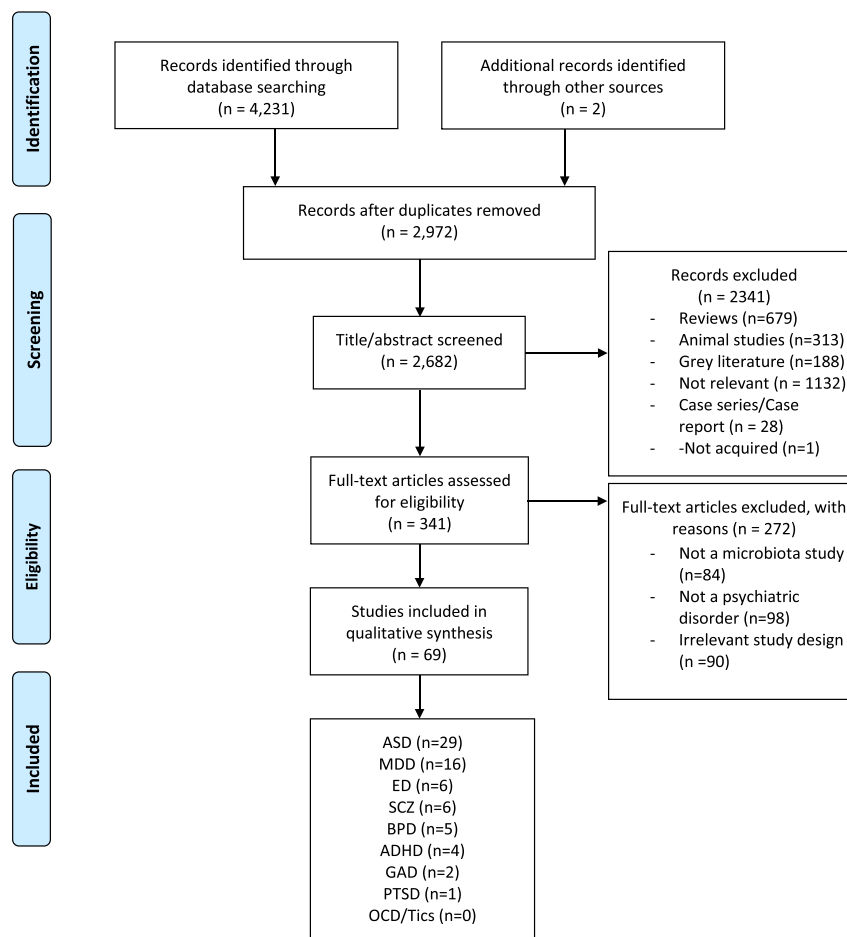


FIGURE 1. PRISMA flow diagram. Attention-deficit/hyperactivity disorder (ADHD), anxiety disorders (ANX), autism spectrum disorder (ASD), bipolar disorder (BPD), eating disorders (ED), major depressive disorder (MDD), tics, obsessive-compulsive disorder (OCD), posttraumatic stress disorder (PTSD), and schizophrenia (SCZ). Color image is available online only with this article at www.psychosomaticmedicine.org.

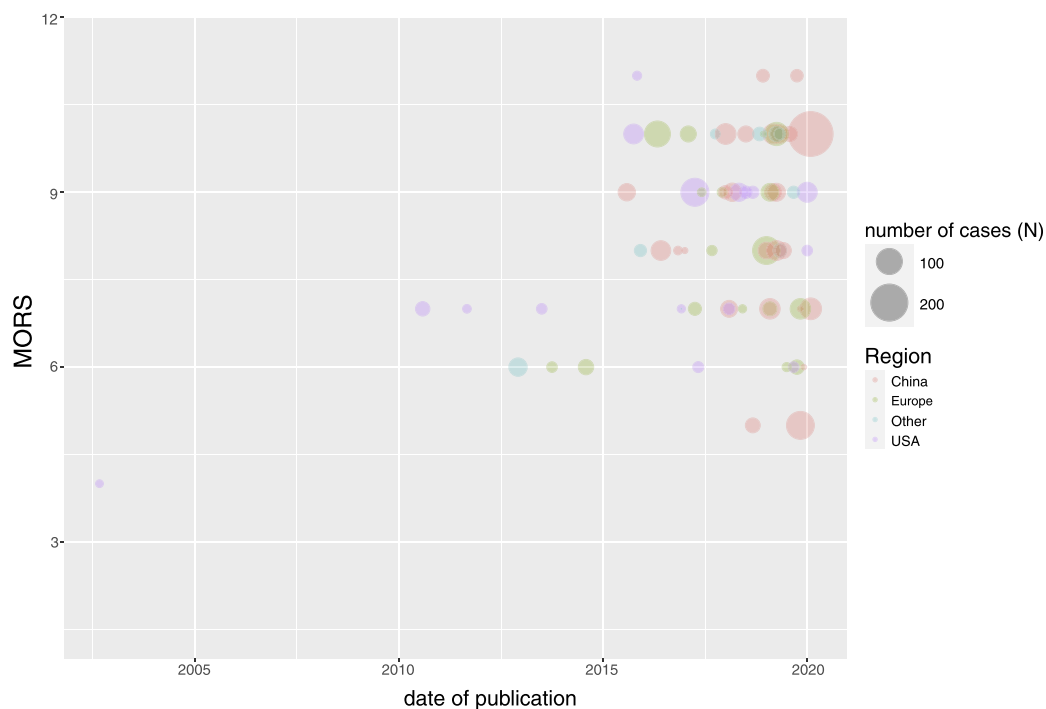


FIGURE 2. Risk of bias visualized with a bubble chart. Modified Newcastle-Ottawa Score Score (MORS) is shown in the y axis and date of publication in the x axis. Each study is represented with a bubble, and the size of the bubble corresponds to the number of cases in the studies. “Region” represents the place where the study was conducted. Color image is available online only with this article at www.psychosomaticmedicine.org.

studies only recruited female cases. For the remaining diagnoses, the sex (male-to-female) ratio was approximately 2:3. Body mass index (BMI) was reported in 44 of 69 studies. Mean BMI for adult cases (ED not included) was 23 kg/m². Eleven of 69 studies specifically reported distribution of race in study participants. Most participants were identified as White, followed by Asian and Black (Supplementary Table 3, <http://links.lww.com/PSYMED/A747>).

Methodological Characteristics

Exclusion Criteria

The 42 different exclusion criteria listed across the studies differ considerably (Supplementary Table 2, <http://links.lww.com/PSYMED/A746>). For example, the required antibiotic-free period before sampling ranged from 2 weeks to 6 months (35,63). A quarter of the studies did not exclude participants who had taken antibiotics recently. Information on smoking and physical activity was collected in 16% and 9% of the studies, respectively (3,27,29,38,47,54,56,62,63,72,73,80,87).

Dietary Assessment

Forty-two percent of the studies assessed the diet of participants in any way, 7% used a food frequency questionnaire, and 10% used food diaries (3,26,28,30,36,51,52,54,58,79,85,89) (Supplementary Table 3, <http://links.lww.com/PSYMED/A747>).

Diagnosis

Eighty-four percent of the studies reported use of diagnostic systems, 43% according to the *Diagnostic and Statistical Manual for Psychiatric Disorders, Fourth Edition (DSM-IV)*, 28% of the

studies according to the *DSM-5*, 7% according to the *International Classification of Disease, Tenth Revision (ICD-10)*, and 6% according to both the *DSM* and *ICD* (90–92). However, only 26% of the studies reported use of a structured diagnostic interview for assessment of diagnoses in study participants (Supplementary Table 3, <http://links.lww.com/PSYMED/A747>).

Sampling and Sequencing

In the majority of studies, samples were frozen at -80°C before extraction (Supplementary Table 4, <http://links.lww.com/PSYMED/A748>). Eighty-six percent of the studies used marker-gene analysis methods, and 9% used metagenome analysis (62,66,67,71,76,81). For the studies that used marker-gene analysis, 16S ribosomal RNA was the most amplified gene, with the hypervariable regions V3–V4 being the most frequently amplified regions (Figure 3A). All amplicons were sequenced with high-throughput sequencing methods using six different sequencing platforms in which MiSeq, Roche 454, and HiSeq were the most used in falling order (Figure 3B). Six studies specifically reported using blank samples during extraction and amplification steps to assess potential contamination of samples or equipment (25,31,33,44,75,82).

Bioinformatic Processing

Fifty-one percent of the studies used QIIME, 19% used Mothur, 7% used R vegan package, and 3% used MEGAN5 for bioinformatic processing (Supplementary Table 4, <http://links.lww.com/PSYMED/A748>). For taxonomic assignment, 49% of the studies used closed-reference picking, 23% used open-reference picking, and 12% used de novo reference picking, whereas the rest of the studies did not report how taxonomic assignments were done or

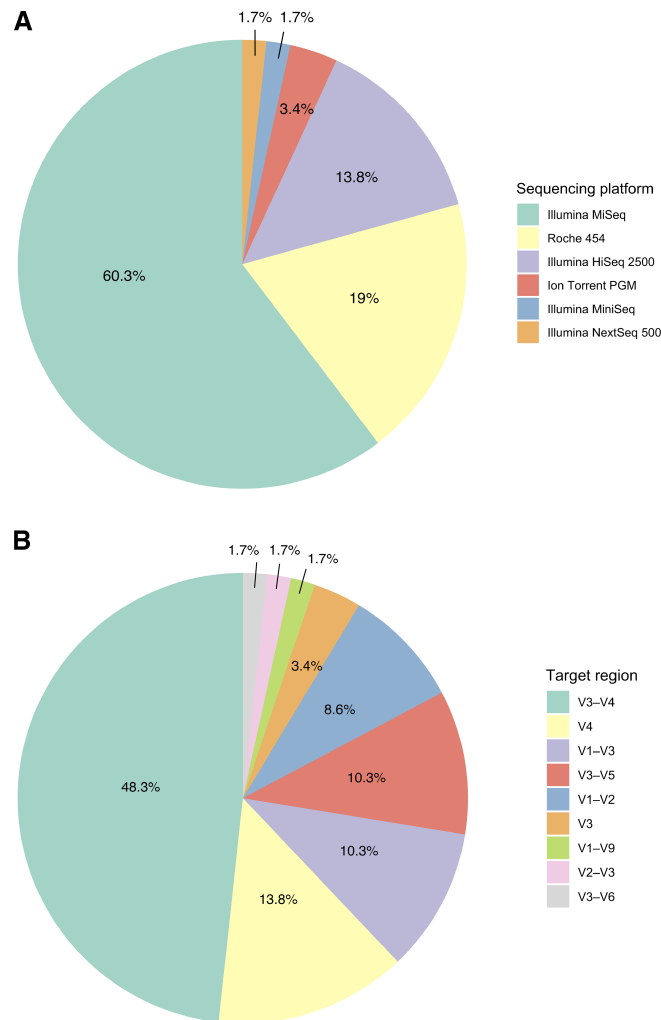


FIGURE 3. Amplification regions and sequencing platforms used in the 16S marker-gene studies. A, Sequencing primers for variable regions (V) shown in percentage. B, Sequencing platform used for high-throughput sequencing shown in percentage. The sequencing platforms and target regions are listed from highest to lowest percentages. Color image is available online only with this article at www.psychosomaticmedicine.org.

had ambiguous information. The most frequently used reference database for taxonomic assignment was GreenGenes (32%) followed by The Ribosomal Database Project (22%) and Silva (16%). Notably, 30% of the studies rarefied and 12% normalized the data set before calculating alpha or beta diversity indices.

Microbial Diversity in Psychiatric Disorders

Alpha Diversity

Among the 57 studies that reported one or several alpha diversity indices, 12% showed increased diversity in cases and 18% found decreased diversity in cases, whereas the majority of studies, 44%, did not detect any significant differences between cases and controls. Twenty-six percent showed mixed results (Supplementary Table 5, <http://links.lww.com/PSYMED/A749>). All alpha diversity indices, separated by disorder, are summarized in Figure 4. Detailed results per disorder are presented in Supplementary Material 3, <http://links.lww.com/PSYMED/A750>.

Beta Diversity

Among the 45 studies that reported beta diversity indices, 67% of the studies detected significant dissimilarity in microbiota composition between cases and controls, 27% showed no significant difference, and 6% had mixed findings (Supplementary Table 5, <http://links.lww.com/PSYMED/A749>). The results are most coherent in ANX, ED, SCZ, and MDD, where all but one study showed significant dissimilarity between cases and controls (40). In ASD, 57% (8 of 14 studies) found significantly dissimilar beta diversity indices between cases and healthy controls (25,30,43,65,69,70,77,89). The results are mixed for BPD and ADHD (Figure 5).

Comparison of Altered Microbiota Among Psychiatric Disorders at Genus Level

To identify patterns in taxonomic differences at genus level among the disorders, we visualized the genus-level alterations in a heat-map (Figure 6). Alterations in the relative abundance of *Bifidobacterium*, *Faecalibacterium*, and *Roseburia* were shared among several

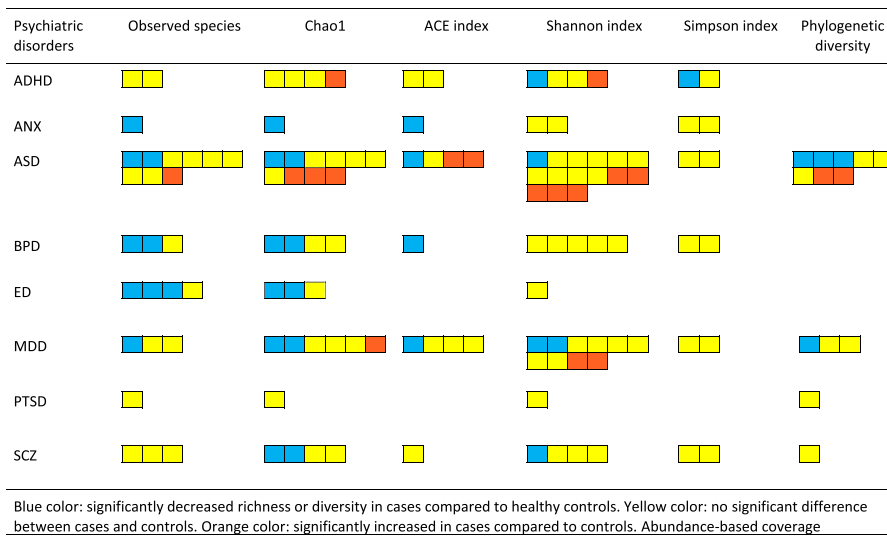


FIGURE 4. Alpha diversity indices reported for each psychiatric disorder. Blue indicates significantly decreased richness of diversity in cases compared with between cases and controls; yellow, no significant difference between cases and controls; and orange, significantly increased in cases compared with controls. ACE = abundance-based coverage estimator; ADHD = attention-deficit/hyperactivity disorder; ANX = anxiety disorder; ASD = autism spectrum disorder; BPD = bipolar disorder; ED = eating disorder; MDD = major depressive disorder; PTSD = posttraumatic stress disorder; SCZ = schizophrenia. Color image is available online only with this article at www.psychosomaticmedicine.org.

psychiatric disorders. Higher abundance of *Bifidobacterium* was found in ADHD, ASD, AN, and MDD (24,27,28,52,58,60,76,88), and lower abundance of *Roseburia* was reported in studies of ASD, AN, BPD, and SCZ (4,26,36,52,63,74,78,80). Furthermore, lower abundance of *Faecalibacterium* was reported in all psychiatric disorders (25,32,40–42,44,45,57,66,74,82,87). There were few replicated findings within each psychiatric disorder, and the abundance of specific genera was contradictory in many cases (Supplementary Material 3, <http://links.lww.com/PSYMED/A750>).

Association Between Gut Microbiota and Clinical Features

Twenty-eight studies reported positive associations between 38 clinical features and microbial alterations in cases (Supplementary Table 5, <http://links.lww.com/PSYMED/A749>). Replicated correlations between studies were generally scarce. *Prevotella* was negatively correlated with depressive symptoms according to Hamilton Depression Rating Scale (47,73). Higher abundance of *Faecalibacterium* was associated with improved quality of life, positively correlated with better sleep, and negatively

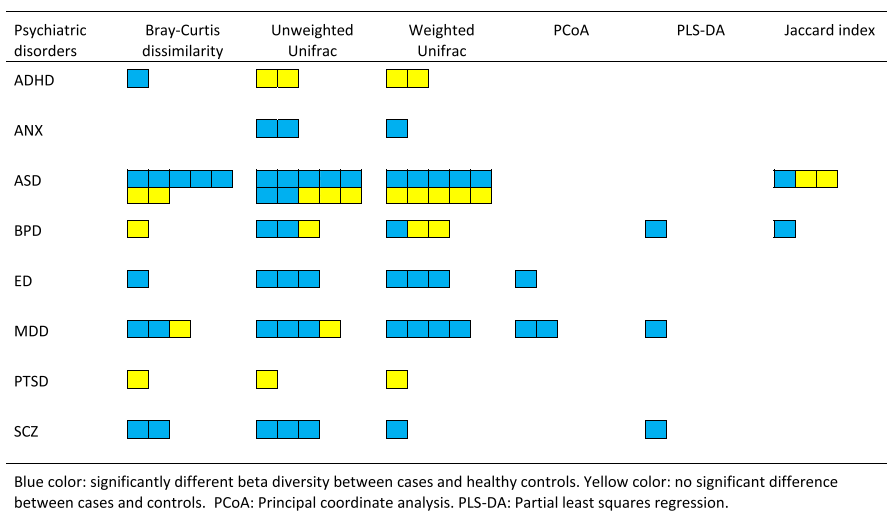


FIGURE 5. Beta diversity indices reported for each psychiatric disorder. Blue indicates significantly different beta diversity between cases and healthy controls; yellow color indicates no significant difference between cases and controls. PCoA = principal coordinate analysis; PLS-DA = partial least squares regression; ADHD = attention-deficit/hyperactivity disorder; ANX = anxiety disorder; ASD = autism spectrum disorder; BPD = bipolar disorder; ED = eating disorder; MDD = major depressive disorder; PTSD = posttraumatic stress disorder; SCZ = schizophrenia. Color image is available online only with this article at www.psychosomaticmedicine.org.

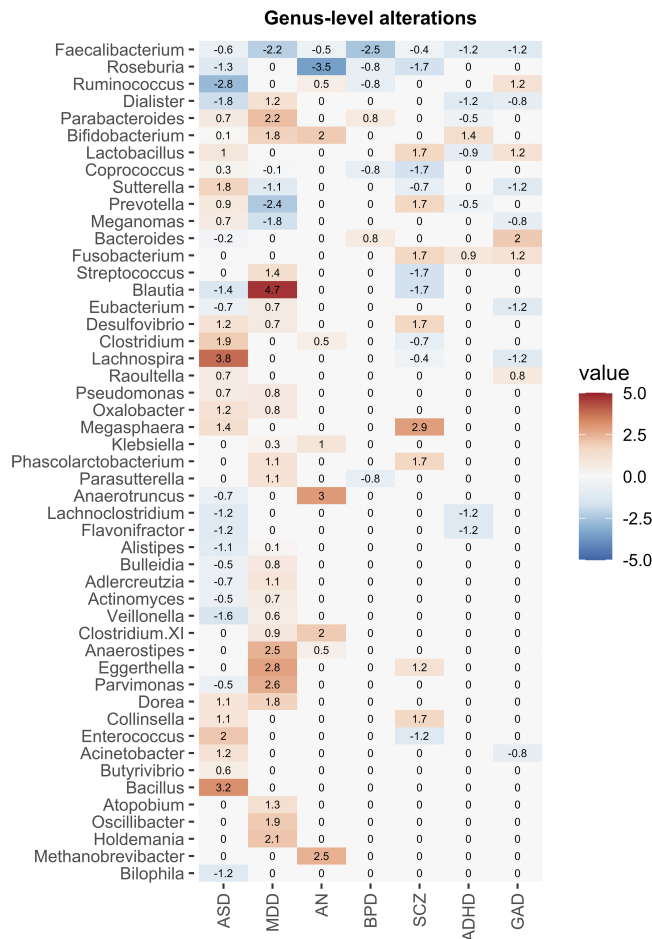


FIGURE 6. Heat-map of the gut microbiota alterations at genus level across psychiatric disorders. Only studies that used 16S rRNA sequencing and genera that were reported in more than one study were included. The values were weighted by sample size in a disorder-specific manner and represent the consistency of the results across the studies. The disorders are listed in order of number of studies included in the heat-map: attention-deficit/hyperactivity disorder (ADHD), Generalized anxiety disorder (GAD), autism spectrum disorder (ASD), bipolar disorder (BPD), anorexia nervosa (AN), major depressive disorder (MDD), tics, obsessive-compulsive disorder (OCD), posttraumatic stress disorder (PTSD), schizophrenia (SCZ). Color image is available online only with this article at www.psychosomaticmedicine.org.

correlated with depressive symptoms, social deficit, and hyperactivity (25,32,40,42,66).

Association Between Short-Chain Fatty Acids and Psychiatric Disorders

In eight studies, levels of short-chain fatty acids (SCFAs) were measured in stool samples (Supplementary Table 5, <http://links.lww.com/PSYMED/A749>). Borgo et al. (26) found significantly decreased levels of total SCFAs, specifically butyrate and propionate, in patients with AN compared with normal-weight controls. Morita et al. (53) supported the finding of decreased levels of propionate in addition to acetate but did not detect significantly lower levels of total SCFA or butyrate. In contrast, Mack et al. (52) found no significant differences in SCFA levels, although the proportion of butyrate was lower in the AN group considering higher levels of branched-chain fatty acids, such as valerate. The only study on SCFAs in stool samples from patients with MDD found no significant difference in SCFA levels in cases compared with healthy controls (3). The results of SCFAs in studies of ASD were ambiguous. Whereas Liu et al. (48) found lower levels of butyrate and acetate, but higher levels of valerate,

Berding and Donovan (25) identified higher levels of butyrate and acetate and propionate. Coretti et al. (30) report higher levels of butyrate, although the levels were within normal range. However, Kang et al. (43) were unable to detect any significant differences in butyrate or propionate concentration between cases and controls.

DISCUSSION

In this systematic review, we explored 69 articles across psychiatric disorders to determine if there are consistent differences in gut microbiota between patients with psychiatric illness and healthy controls. Our findings were limited by the methodological differences at various levels across the studies. Here, we summarize the findings, discuss the methodological differences, and provide recommendations for improving microbiota studies in psychiatric disorders.

Summary of Evidence

Alpha and Beta Diversity Between Psychiatric Disorders and Controls

In summary, either there was no significant difference in alpha diversity between patients with psychiatric disorders and controls or

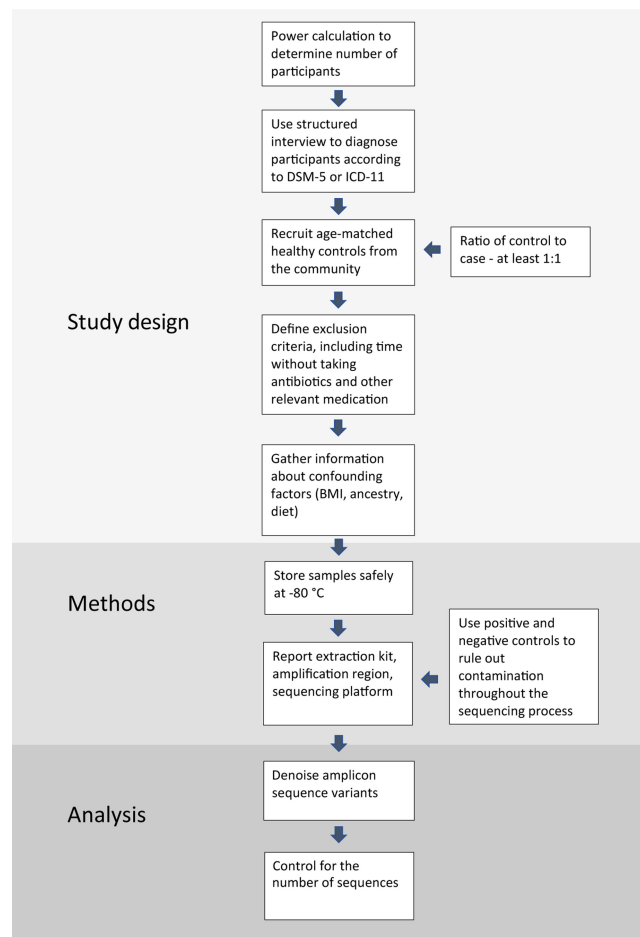


FIGURE 7. Recommendations on how to conduct a microbiota study of psychiatric disorders. DSM-5 = Diagnostic and Statistical Manual for Psychiatric Disorders, Fifth Edition; ICD-11 = International Classification of Disease, 11th Revision; BMI = body mass index. Color image is available online only with this article at www.psychosomaticmedicine.org.

the results were ambiguous. Across all reviewed disorders, the most consistent alpha diversity results emerged in AN, with three of four studies reporting a significant decrease in species richness in patients with AN (36,45,54). AN is associated with prolonged caloric restriction. Decreased alpha diversity in patients with AN could partly reflect prolonged restricted energy consumption. Dietary components such as carbohydrates and proteins nourish microbes in the gastrointestinal tract. When there is low dietary intake, like in AN, it may create a nonconductive environment for some microbes, subsequently affecting diversity (93).

In the majority of studies reviewed, beta diversity analysis showed a separation between patients with a psychiatric disorder and healthy controls; however, there is only limited consensus on which bacteria that differ in abundance between cases and controls. The most replicated findings are lower abundance of butyrate-producing genera, *Roseburia* and *Faecalibacterium*, and higher abundance of commensal bacteria, *Bifidobacterium*.

Decreased Abundance of Butyrate-Producing Genera in Psychiatric Disorders

The abundance of *Roseburia* was decreased in four psychiatric disorders (ASD, AN, BPD, SCZ). *Roseburia* includes five species that produce SCFAs, especially butyrate, and is associated with

metabolic disorders such as type 2 diabetes and obesity (94,95). We also observed decreased abundance of *Faecalibacterium* across all psychiatric disorders. Interestingly, the abundance of *Faecalibacterium* is associated with various health benefits, such as less depressive symptoms, and better sleep, and improved quality of life (25,32,40,42,66). *Faecalibacterium* has a sole known species, *Faecalibacterium prausnitzii*, which constitutes between 5% and 15% of the human gut microbiota. It has anti-inflammatory properties and promotes intestinal barrier function through modulation of tight-junction protein expression and is one of the main butyrate producers (96,97). Depletion of *F. prausnitzii* is evident in inflammatory bowel diseases and even in patients with COVID-19 (98,99). The abundance of *F. prausnitzii* was inversely correlated with disease severity of COVID-19 (100).

Butyrate, one of the most abundant SCFAs, is a metabolite from the bacterial fermentation of dietary fibers. It is a crucial energy source for colonocytes and has anti-inflammatory properties of its own (101). In mice, oral administration of sodium butyrate was shown to have antidepressive effects in forced swim and tail suspension tests (102). In addition, supplementation of sodium butyrate can attenuate social deficits and decrease repetitive behavior (103). It has been suggested that butyrate could influence the brain monoaminergic pathway through modulation of the

histone deacetylase inhibitor, thus affecting gene expression (104). In patients with AN, lower levels of butyrate compared with healthy controls have been documented (26,52,53,105). The mechanistic effect of butyrate in psychiatric disorders requires further inquiry.

Increased Abundance of Commensal Genera *Bifidobacterium* in Psychiatric Disorders

Bifidobacterium, a genus of commensal bacteria colonizing the gut, was elevated in several psychiatric disorders. There are more than 50 different known strains of *Bifidobacterium*. Because of various ascribed health benefits (106), different strains of *Bifidobacterium* are used as probiotics (107), despite insufficient scientific evidence (108). In fact, gut commensal bacteria may have complex effects on the host depending on the host genetics, environmental factors, and the overall gut microbiota composition. Thereby, beneficial bacteria can turn pathobiotic in some circumstances (109). Deeper sequencing at the species level, functional profiling, and mapping diet and host genetics might take us closer to elucidating the role of altered levels of *Bifidobacterium* in psychiatric disorders.

Recommendations

Most reviewed studies suffered from methodological limitations that affect the generalizability of the findings. Prominent methodological issues included small sample size, differences in patient selection and diagnosis, failure to exclude or account for confounding factors, and shortcomings in bioinformatics analysis. Therefore, we propose recommendations to improve the quality and comparability of future studies (Figure 7).

Statistical Power and Case/Control Ratio

None of the studies included in this systematic review reported any kind of power calculation. Assessing the number of study participants needed to avoid type II error is fundamental in all scientific disciplines. However, in microbiome studies, power analyses remain challenging because of unknown true effect size and unknown composition of the microbiome in case and control groups. Nevertheless, several tools exist for calculating power in microbiota studies, for example, the HMP package or the micropower package (110). Importantly, power increases significantly by stratified sampling of controls on critical confounding factors, such as age, sex, and BMI. In addition, it is generally recommended that the ratio of controls to cases is at least 1:1 or higher (111).

Diagnostics

A minority of studies adopted validated structured diagnostic interviews for cases and controls (112). The recommendation is to use structured diagnostic interviews to assess diagnosis and comorbidities in cases and to rule out psychiatric illness in healthy controls.

Confounding Factors

There are two main groups of confounders in microbiota research: demographic variables including age, sex, ethnicity, and BMI, and environmental factors that include diet, medication, and life-style factors.

Age

The gut microbiota is not static and changes throughout life (21). In addition, aging has been linked to gain of disease-associated

gut microbes in many diseases, such as diabetes and colorectal cancer (113). Therefore, we recommend frequency-based matching of controls based on important confounding factors, such as age.

Sex

Women have been reported to have higher alpha diversity than men, and microbial composition seems to vary significantly between sexes after puberty (114). In this review, AN and ASD groups have the most skewed sex ratios, underscoring the importance of matching the sex of cases to controls or adjusting for this confounding factor in disorders with considerable sex imbalances (115).

Ancestry and Geography

Gut microbiota may also be influenced by ancestry and geographical location (116–118). For example, healthy Black women have higher abundance of *Bacteroides* compared with White women (119). Because we cannot assume that all participants belong to the predominant ancestry group of that country, ancestry and geographic location of the study participants are important confounders to report.

Body Mass Index

Gut microbial diversity and composition have been shown to be associated with BMI, even after controlling for confounding factors such as sex and age (120,121). BMI is typically lower in AN, for example, which could be associated with the lower alpha diversity in the studies we reviewed. Given that BMI is known to deviate from the general population in individuals with many mental illnesses, BMI should be taken into consideration in microbiota studies of psychiatric disorders (122).

Diet

Microbial community structure and activity can be influenced by both short-term changes in diet (123) and long-term dietary patterns (93). In the studies reviewed here, only ~40% performed dietary assessments using a variety of approaches such as habitual food frequency questionnaires, 24-hour food recall, conventional macronutrient and micronutrient profiles, for example. Lack of standardized and comparable methods to assess the effects of food intake on the gut microbiota remains a limitation in microbiome research (124).

Medication

Antibiotics have a major impact on gut microbiota, and it can take up to 6 months for the gut microbiota to recover (125). Although requiring 6 months without antibiotics before inclusion could curtail the number of eligible participants in a study, fewer than 3 months without antibiotics before participation is not advisable. Of particular interest to this review, microbiota alterations associated with antipsychotic medication have been described in both animal and human studies (126,127). However, excluding patients on antipsychotic medication in studies of psychotic or BPDs might exclude most of eligible patients, whereas controlling for medication would be a more tenable option. Finally, when conducting a study on microbiota in psychiatric disorders, it is also important to document and, if possible, adjust for unusual medications used by individuals with specific disorders such as laxatives in AN and commonly abused substances from nicotine to opiates (128,129).

Bioinformatic Processing

It has been shown that differences in methodology regarding extraction, amplification, sequencing, and library preparation are sources of confounding. For example, the variation in microbiota profiles was greater in the same individual using different amplification regions than variation in stool samples from different individuals using the same amplification regions (130). As a result, it is recommended to harmonize amplification regions across studies. Further guidelines on methodology for microbiota studies have already been covered extensively (131–133). Here we highlight two aspects of data analysis that we came across in several studies in this systematic review.

Next-generation sequencing (NGS) entails unequal library sizes. Rarefying is commonly adopted to estimate uncertainty in NGS count data by selecting a minimum library size, discard libraries that are smaller than the set size, and subsample the remaining libraries without replacement. However, there are crucial flaws with this approach. It lowers power by discarding samples that cannot be classified, and it does not account for overdispersion, thus providing inferior sensitivity compared with an infinite mixture model, such as negative binomial or Gamma-Poisson (134).

It is customary to use operational taxonomic unit (OTU) picking where reads are clustered according to a set dissimilarity threshold that is arbitrarily chosen. An alternative is amplicon sequence variants, which infers sequences exactly instead of constructing OTUs. It not only offers resolution down to a single nucleotide difference; more importantly, the results are comparable between studies without sacrificing reads when using closed-reference OTU picking with reference databases. Comparable in performance to amplicon sequence variants using DADA2 is denoising with Deblur (135). In summary, ecological validity can be seriously hampered by both rarefaction and OTU picking, which has pivotal implications for downstream analysis, for example, microbiota diversity.

Lastly, we encourage researchers to be selective in their hypotheses testing and using carefully considered microbiota analysis. Preregistration of study design and analysis methods should be mandatory.

Future Perspectives

Large sample sizes are critical for adequate power, but it requires large-scale collaboration and proper funding. For now, one way to ramp up sample size is to use an open-source database depository such as Qiita (136). It allows sharing and reusing data sets from previous studies, in addition to performing new analyses according to current best practices. Although the gut microbiota is fairly stable during adulthood, its diversity and composition can change with diet, exercise, use of antibiotics, and so on. Therefore, it is important to collect longitudinal data to determine whether the differences in microbiota between patients with a psychiatric illness and controls are a state or a trait (2). Finally, as the price drops for shotgun metagenomic sequencing, the popularity of marker-gene sequencing might be diminished. Shotgun metagenomics has the benefit of higher resolution, down to the species level, enabling a more specific taxonomic and functional characterization of the gut microbes.

Limitations

Several limitations of our systematic review should be considered. First, by excluding interventional studies, we may have overlooked

some contributions to the knowledge base. Second, our review was limited to English-language reports. Third, although a strength of our review is the comprehensive review across psychiatric disorders, it also introduced complexities as the psychiatric illnesses studied naturally vary by sex and age (e.g., samples from patients with AN were skewed toward women, and samples from patients with ADHD were skewed toward young men), meaning that it was not always possible to disambiguate diagnosis from age and sex. In addition, comorbidities between psychiatric disorders are common. It is possible that unmeasured comorbidities increased similarity of findings across different psychiatric disorders. Future studies should consider and control for co-occurring presentations whenever possible.

Conclusions

Our review suggests that the study of the intestinal microbiota in psychiatric disorders is characterized by minimal cohesion and few reliable replications. In part, this reflects the stage of the field as methods are still being optimized and standardized. Given this context, the limited evidence suggests no consistent differences in alpha diversity between patients with psychiatric disorders (AN being an exception) and healthy controls. However, we found more consistent differences in beta diversities, indicating dissimilar microbiota composition between patients with a psychiatric disorder and healthy controls. The most replicated findings at genus level include decreased abundance of SCFA-producing microbes, such as *F. prausnitzii* and *Roseburia* species, and increased abundance of *Bifidobacterium* in patients with psychiatric disorders, which provide grounds for further functional and mechanistic investigations. Finally, to improve study quality, collaboration between psychiatrists and microbiologists is imperative in planning and executing microbiome studies in psychiatric disorders.

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