Chapter 21

Microbiome and bipolar disorder

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21.1 The microbiota-gut-brain axis: a new approach in bipolar disorder biomarkers

21.1.1 Introduction

In the classical bowel toxemia theory, the intestines were often cited as the locus of the systemic self-poisoning process. In this line, Hermann Senator (1834−1911) speculated already in the 19th century that "self-infection" originating in the intestines could cause disease elsewhere in the body, including mental disturbances such as acute delirium (Noll, 2004). In the last 15 years, research on how gut microbiota contributes to normal physiological processes and how it might influence disease predisposition has exponentially increased. By now, there is some evidence on its involvement in the pathogenesis of a variety of somatic diseases, such as allergy, asthma, or Crohn's disease (Alemao et al., 2021; Barcik, Boutin, Sokolowska, & Finlay, 2020; Serena et al., 2020). Moreover, certain alterations in microbiota composition might have the potential to be used as a maker of treatment response or clinical prognosis in some somatic diseases (Hyams et al., 2019; Peled et al., 2020). For instance, a recent multicenter study reported an association between particular changes in microbiota and a higher risk of death following transplantation in patients undergoing allogeneic hematopoietic-cell transplantation (Peled et al., 2020).

Gut microbiota and its implications in mental health have also regain prominence among researchers, as gut microbiota is appearing as a key moderator in the well-known bidirectional communication between the gastrointestinal tract and the central nervous system (CNS) (Iannone et al., 2019). In this context, researchers have developed the concept of microbiota-gut-brain axis (MGBA), which translates the potential role of microbiota in the

Biomarkers in Bipolar Disorders. DOI: https://doi.org/10.1016/B978-0-12-821398-8.00004-7

neurobiology of neuropsychiatric illnesses, including bipolar disorder (BD). In particular, it has been suggested that alterations in the composition of gut microbiota might influence physiological pathways thought to be involved in the pathogenesis of affective disorders, such as the dysregulation of the hypothalamic−pituitary−adrenocortical (HPA) axis or the activation of the immune system (Iannone et al., 2019).

However, gut microbiota might not only help us to deepen our understanding on the etiopathogenesis of affective disorders, but it might also present as a potential biomarker for these disorders, as suggested by the fact that several clinical studies have reported differences in gut microbiota composition between subjects with affective disorders and healthy controls (Vinberg et al., 2019).

The aim of this chapter is, on one hand, to give an overview of the MGBA and the available tools to study this axis and, on the other hand, to outline how alterations in gut microbiota composition might serve as biomarkers for BD.

21.2 The microbiota-gut-brain axis

The human gastrointestinal tract houses nearly 10^4 microorganisms, including bacteria, helminthic parasites, or viruses (Jaggar, Rea, Spichak, Dinan, & Cryan, 2020). These microorganisms define the gut microbiota (Jaggar et al., 2020). The term *microbiome* refers to the genes of these microorganisms, although it is commonly used to refer to the microorganisms themselves (Allaband et al., 2019). Among bacteria, the most prominent phyla are Bacteroidetes, Firmicutes, Proteobacteria, and Actinobacteria (Hugon et al., 2015). They comprise around 90% of gut microbiota (Consortium, 2012; Hugon et al., 2015).

Gut microbiota composition first settles at birth, where it can be influenced by a series of factors such as mode of delivery (vaginal vs cesarean) or type of feeding (breast vs formula) (Cryan et al., 2019) (Fig. 21.1). In the following years of life, gut microbiota composition shifts toward and adult configuration shaped by the gender and the genetics of the host or his/her geography (Hugon et al., 2015; Lynch & Pedersen, 2016). This microbiota signature is unique for every individual, although gut microbiota is a dynamic system whose density and composition can be further influenced by external factors such as medication use—specially antibiotics—diet or infections (Lynch & Pedersen, 2016).

Human gut microbiota is involved in a number of biological processes in the host. For instance, it contributes to the maturation of the immune system and regulates intestinal endocrine functions (Lynch & Pedersen, 2016). With regard to the CNS, gut microbiota also regulates neurologic signaling and biosynthesizes neurotransmitters (Lynch & Pedersen, 2016). At the same time, the CNS communicates through the autonomic nervous system with different intestinal targets, such as muscle layers and gut mucosa, and

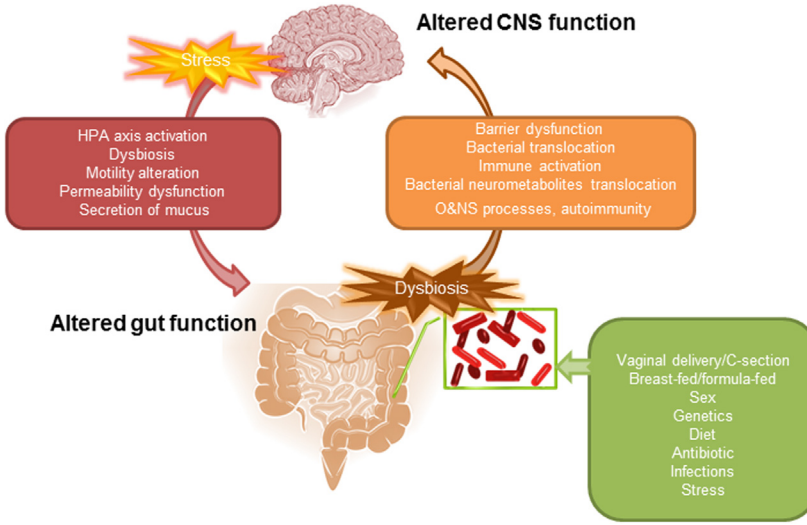

FIGURE 21.1 Bidirectional modulation of the microbiota-gut-brain axis. *Gut microbiota composition can be influenced by a series of factors, including the gender and genetics of the host, mode of delivery (vaginal vs cesarean), type of feeding when born (breast vs formula), diet, antibiotics, infections, or stress. Stress activates the HPA axis, increasing circulating levels of cortisol and leading to a change in the composition of commensal bacteria (dysbiosis). Dysbiosis induces an increase of epithelial permeability and facilitates the translocation of enteric pathogens (leaky gut). In turn, leaky gut contributes to the translocation of neurometabolic products generated by the microbiota, such as short chain fatty acids, tryptophan, dopamine, GABA and serotonin, that might be able to affect the brain. Besides, bacterial translocation can induce a systemic immune and inflammatory response, while structures of the bacterial wall mimicking components of the brain may elicit an autoimmune response against the brain. This inflammatory response may further activate the HPA axis, potentiating the harm to the brain and the epithelial barrier. Finally, CNS communicates through the autonomic nervous system with different intestinal targets such as muscle layers and gut mucosa, thus modulating motility, immunity, permeability, and secretion of mucus, which might contribute to the altered gut function.*

modulates motility, immunity, permeability, and secretion of mucus (Iannone et al., 2019). Also, much of the sensory input from the gut is transmitted to the brain by the afferent fibers of the vagus nerve (Cryan et al., 2019). This interaction between brain, gut, and gut microbiota is integrated in the MGBA, which consists of a combination of neural (the autonomic nervous system and the enteric nervous system), endocrine, immune, metabolic, and cellular pathways that interact among them in an antagonistic and synergic manner (Cryan et al., 2019).

21.2.1 Consequences of microbiota-gut-brain axis alteration

Under normal physiological conditions, the gut microbiota lies in a stable equilibrium at the interface between the internal and external

environment of the gut, where it plays several roles including helping in the development of the intestinal epithelial barrier and protecting against pathogens (Chan, Estaki, & Gibson, 2013; Forbes, Van Domselaar, & Bernstein, 2016). Also, under normal physiological conditions, the intestinal mucosal barrier includes both secretory and physical preventive measures against the translocation of microbes and other proinflammatory molecules—such as toxins or antigens—from the luminal environment into the sterile mucosal tissues and circulatory system (Suzuki, 2013). However, several factors can disrupt the ecology and function of microbiota, such as antibiotic use, modern diet, or psychological and physical stress (Cryan et al., 2019; Lynch & Pedersen, 2016). This alteration of the microbiota composition is called dysbiosis (Forbes et al., 2016). Importantly, dysbiosis can disrupt the intestinal barrier and lead to an impaired intestinal permeability (the so-called leaky gut), which allows the translocation of commensal bacterial and its products, including neurometabolites, neuropeptides, or particles such as lipopolysaccharides (LPS) (Groschwitz & Hogan, 2009). Bacterial translocation can induce proinflammatory pathways and increase cytokine levels, such as interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα), or interferon-y (IFN-γ), with the ensuing immune inflammatory activation (Cryan et al., 2019; Fung, 2020). Moreover, TNF-α may activate the HPA axis, which in turn can modulate the immune response (Misiak et al., 2020). This proinflammatory state aggravates the loss of epithelial barrier function (Groschwitz & Hogan, 2009). On the other hand, inflammation leads to tryptophan depletion, the main precursor of serotonin (Charlotte Hunt et al., 2020). Immune activation and inflammation also lead to the production of reactive oxygen and nitrogen species (ROS and RNS, respectively) such as nitric oxide (NO) (Slyepchenko et al., 2016). Importantly, this increased ROS and lipid peroxidation may further weaken the mucosal barrier (Groschwitz & Hogan, 2009). Autoimmune processes can likewise develop after LPS-induced oxidative and nitrosative stress (O&NS), which denatures the structure of endogenous molecules rendering them immunogenic and increasing plasma levels of IgM and IgA (Maes et al., 2013; Simeonova et al., 2020).

In conclusion, as a consequence of dysbiosis, leaky gut and bacterial translocation, there is an activation of several proinflammatory mediators and O&NS processes. Moreover, the inflammatory response—among other factors—contributes to increase the damage on epithelial barrier integrity, thus perpetuating the cycle.

21.2.2 Microbiota-gut-brain axis and mental health

While the exact mechanism through which alterations in the MGBA may influence mental disorders remains unknown, the most extended theory is that alterations in the intestinal epithelial permeability mediated by

enteropathogenic bacteria or stress contribute to bacterial translocation and migration of metabolites and neurometabolites generated by gut microbiota (Cryan et al., 2019; Fung, 2020). Also, bacterial translocation triggers a systemic immune inflammatory response and can develop cross immune reactions against components of the brain (Petta, Fraussen, Somers, & Kleinewietfeld, 2018) as it has been demonstrated that gut microbiota contains microorganisms with molecular mimicry to neuropeptides involved in the regulation of motivated behavior, mood, and emotion, which can generate cross-reacting autoantibodies (Fetissov & Déchelotte, 2011). Furthermore, bacteria are able to generate neurometabolic products and neuropeptides (Cryan et al., 2019). They can produce gamma-aminobutyric acid (GABA) (Barrett, Ross, O'Toole, Fitzgerald, & Stanton, 2012) dopamine, noradrenaline, and serotonin (Chong et al., 2019). Microbiota can also create valeric acid as its main metabolic end product, a homolog of the neurotransmitter GABA (Naseribafrouei et al., 2014). These changes in neurotransmitters might also influence brain functions. Furthermore, the gut microbiota regulates monocyte trafficking to the brain and the function of CNS-resident immune cells, further contributing to neuroinflammation (van de Wouw, Boehme, Dinan, & Cryan, 2019).

All these processes, together with the activation of neural pathways by gut microbiota through the enteric nervous system or by modulation of vagal afferents (Bravo et al., 2011; Goehler et al., 2005; Wang, Ishima, & Zhang, 2020) are thought to have an impact on brain functions, change behavior and modify subjective experiences.

21.2.3 Available tools to explore the microbiota-gut-brain axis

Information regarding the MGBA can come from clinical or animal studies. Fig. 21.2 is a schematic representation of the different kind of studies and analysis techniques that contribute to the understanding of the MGBA. Tools to assess the influence of gut microbiota in the brain or in behavior differ in humans and rodents. In animal studies, objective changes in behavior, histopathology, and immunochemistry are available tools, while functional neuroimaging or objective/subjective scales assessing psychiatric symptoms are often used in clinical studies.

Many of the animal studies are performed in germ-free mice (GFM), also known as gnotobiotics, which are mice who are removed from the womb of their mothers through a careful surgical procedure to avoid exposing them to the microorganisms present in the vagina and skin of the mother (Kennedy, King, & Baldridge, 2018). Later, the animal is raised in a sterile cage and it is only exposed to food, water, and other equipment that have been also sterilized. GFM provide the possibility to assess the effect of particular bacteria in the host and to study how the absence of gut microbiota during development alters brain function and development (Braniste et al., 2014; Kennedy et al., 2018). Other animal studies explore the effect of human fecal

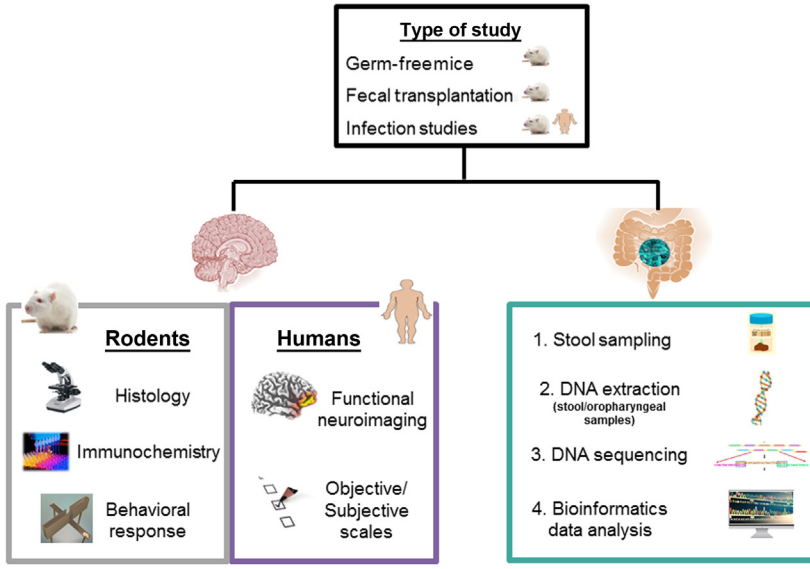

FIGURE 21.2 How have researchers expanded their knowledge about the microbiota-gut-brain axis? *Different types of studies are useful to investigate the MGBA, including GFM studies, fecal transplantation studies, and infection studies. The former two, though, are only performed in animals, whereas infection studies can be performed both in human and animals. Measurements tools to assess the potential impact of gut microbiota on the brain/behavior differ in rodents and human studies. Rodent studies examine behavior changes, investigate brain modifications by histology and immunochemistry. On the contrary, human studies assess subjective response, changes in brain functions by functional neuroimaging. For the characterization of gut microbiota from stool samples, genomic sequencing—for instance, with 16S ribosomal RNA gene sequencing or metagenomic approaches—coupled with computational pipelines are frequently used (Allaband et al., 2019).*

transplantation on GFM. Results from these kinds of studies have shown, for instance, that GFM can exhibit anhedonia and anxiety-like behavior after the transplantation of fecal microbiota from patients with depressive symptoms (Kelly et al., 2016). Infection studies—the administration of antimicrobials or probiotics—can be performed both in humans and rodents (Kennedy et al., 2018). Administration of probiotics may be useful to evaluate the impact of specific pathogens on the host. In turn, antibiotics can alter microbiota composition so their administration might help to assess alterations in behavior mediated by dysbiosis.

When it comes to the study of gut microbiota, great advances have been seen in recent years in the tools used for its analysis. Targeted approaches such as 16S ribosomal RNA (a component of the 30S ribosomal subunit found in prokaryotes) gene sequencing are commonly used for bacterial community profiling (Song, Lee, & Nam, 2018). This method uses selective amplification followed by sequencing the 16S rRNA gene that is found in all bacteria and

archaea (Clavel, Lagkouvardos, & Hiergeist, 2016). It allows for the identification of components of bacteria in the gut microbiota that could not previously be cultured, enabling a better definition of the composition and diversity of the gut microbiota (Clavel et al., 2016). Another advantage of this method is that there are several databases available which integrate 16S rRNA amplicon data across multiple samples, such as the bioinformatic pipelines Quantitative Insights Into Microbial Ecology (QIIME) or metametaDB (Clavel et al., 2016). Metagenomic approaches are another commonly used method to characterize the gut microbiota (Song et al., 2018). Metagenomics consists in the study of genetic material isolated directly from patient samples, such as fecal samples, using high-throughput techniques. It is also referred to as shotgun metagenomics as it sequences the entire genome in fragments instead of amplifying specific gene regions (Song et al., 2018). Culturomics is an alternative high-throughput technique for the identification of bacterial species that uses multiple culture conditions, MALDI-TOF (matrix-assisted laser desorption/ionization—time-of-flight) mass spectrometry and, if necessary, 16S rRNA sequencing (Lagier et al., 2018). MALDI-TOF mass spectrometry allows the identification of proteins through their mass and their charge number (Lagier et al., 2018). The multiple culture conditions provided by culturomics have helped to identify unknown bacteria that inhabit the human gut (Lagier et al., 2018).

21.3 Microbiota-gut-brain axis and bipolar disorder: gut microbiota as a potential biomarker for bipolar disorder

BD is a chronic psychiatric disorder with a lifetime prevalence of 2.4% (Vieta et al., 2018). One major challenge in BD is its clinical heterogeneity, which often leads to underdiagnosis or misdiagnosis as another psychiatric condition, most commonly depressive disorder (Musliner & Østergaard, 2018). Furthermore, BD is associated with important disability, especially among those patients with a more recurrent course (Carvalho, Firth, & Vieta, 2020). An early diagnosis and choice of the optimum treatment are essential to achieve better illness outcomes. For this reason, researchers are on the look for objective markers that could help to improve diagnosis and guide treatment choice (Frey et al., 2013). Changes in gut microbiota in individuals with BD are being examined as potential candidates to become diagnostic and treatment biomarkers. Table 21.1 describes the main findings of the studies evaluating gut microbiota composition in BD and their potential use in the biomarker field.

21.3.1 Gut microbiota as diagnostic biomarker for bipolar disorder

Several studies have investigated differences in gut microbiota composition between subjects with BD and healthy controls. The most prominent and consistent finding to date has been the presence of decreased levels of

TABLE 21.1 Main findings of articles assessing gut microbiota in bipolar disorder and their potential use as biomarkers.

Authors, year	Main findings	Type of candidate biomarker
Evans et al. (2017)	BD versus HC:¬↓ *Firmicutes* genus (*Ruminococcaceae* family and *Faecalibacterium* genus)	Diagnostic biomarker
Flowers et al. (2017)	¬↓ species diversity in female BD patients treated with AAP	Treatment biomarkers
Guo et al. (2018)	BD versus HC:¬↑ microbial diversity (> manic)	Diagnostic biomarker
	Mania versus depression:¬↑ *Escherichia coli* and *Bifidobacterium adolescentis* (mania)¬↑ *Stercoris* (depression)	State biomarker
Aizawa et al. (2018)	BD versus HC:¬No significant between-group differences in *Bifidobacterium* or *Lactobacillus* counts	–
Coello et al. (2019)	BD versus HC versus BDR:¬No difference in bacterial diversity or richness between groups¬*Flavonifractor* ↑ *BD; BDR = HC*	Diagnostic biomarker
Hang Rong et al. (2019)	BD and MDD versus HC:¬↑ *Firmicutes and Actinobacteria* phylum¬↓ *Bacteroidetes* BD versus HC:¬↑ *Proteobacteria*	Diagnostic biomarker
	BD versus MDD:¬↓ *Prevotellaceae, Bifidobacterium*¬↑ *Fusobacteriaceae, Escherichia blattae* and *Klebsiella oxytoca*	Differential diagnosis biomarker (BD vs MDD)
Hu et al. (2019)	BD versus HC:¬↑ *Bacteroidetes* phylum (*Parabacteroides, Bacteroides* genus and *Halomonas* genus)¬↓ *Firmicutes* phylum (*Roseburia, Faecalibacterium,* and *Coprococcus* genus)	Diagnostic biomarker
	BD II versus BD I¬↑ *Ruminococcus*	Differential diagnosis biomarker (BD-II vs BD-I)
	BD untreated versus treated with QTP:¬↑ *Klebsiella* and *Veillonella*	Treatment biomarkers
Lu et al. (2019)	BD versus HC:¬↑ *Bacteroides* and *Prevotella* genus, *Enterobacter* genus, *Atopobium* Cluster, and *Clostridium* Cluster *IV (*including *Faecalibacterium prausnitzii)*	Diagnostic biomarker
	BD patients with QTP:¬↑ *Eubacterium rectale, Bifidobacteria*	Treatment biomarkers

(*Continued*)

TABLE 21.1 (Continued)

Authors, year	Main findings	Type of candidate biomarker
McIntyre et al., (2019)	BD versus HC:¬↓ microbiota diversity	Diagnostic biomarker
	BD-II versus BD-I:¬↑ Collinsella	Differential diagnosis biomarker (BD-II vs BD-I)
Painold et al. (2019)	BD versus HC:¬↓ *Firmicutes* phylum (*Ruminococcaceae* family and *Faecalibacterium* genus)¬↑ *Actinobacteria* phylum (*Coriobacteriaceae* family)	Diagnostic biomarker
	Euthymia versus Depression:¬↑ *Enterobacteriaceae* (moderate depression)¬↑ *Clostridiaceae family and Roseburia genus* (recovered patients)	State biomarker
Rhee et al. (2020)	BD versus MDD:¬↓ *Prevotella* 2, *Ruminococcaceae* UCG-002 genera	Differential diagnosis biomarker (BD vs MDD)

Abbreviations: *BD,* bipolar disorder; *HC,* healthy controls; *BDR,* BD relatives; *MDD,* major depressive disorder; *AAP,* atypical antipsychotic; *QTP*: quetiapine.

Faecalibacterium genus, a gram-positive butyrate-producing gut bacterium member of Firmicutes phylum (Ferreira-Halder, Faria, & Andrade, 2017) in BD samples (Evans et al., 2017; Hu et al., 2019; Painold et al., 2019). Similar findings have been reported in studies performed in subjects with depressive disorders (Sanada et al., 2020; Vindegaard, Speyer, Nordentoft, Rasmussen, & Benros, 2020) or multiple sclerosis (Cantarel et al., 2015) which might indicate that this alteration is not unique to BD. Higher counts of Actinobacteria phylum (Painold et al., 2019; Rong et al., 2019) and *Bacteroides* (Hu et al., 2019; Lu et al., 2019), *Parabacteroides* (Hu et al., 2019), *Prevotella* (Lu et al., 2019), *Halomonas* (Hu et al., 2019), and *Enterobacter* (Lu et al., 2019) genus in BD compared to healthy controls have also been described. *Escherichia*, *Klebsiella*, *Streptococcus, Clostridium*, and *Oscillibacter* levels have been reported to be increased in subjects with BD and major depressive disorder (MDD) in an acute depressive episode in comparison to healthy controls (Rong et al., 2019). The main metabolic end product of *Oscillibacter* is valeric acid, which structurally resembles neurotransmitter GABA, so it has been hypothesized that *Oscillibacter* might modulate depressive behavior through GABA signaling (Naseribafrouei et al., 2014).

In a sample of newly diagnosed subjects with BD, the presence of *Flavonifractor* genus was associated with an odds ratio of 2.9 for having

BD, although the authors warn on the potential role of smoking as a driver of their findings (Coello et al., 2019). Another study in drug-free or drug-naïve patients with BD showed an elevated *Faecalibacterium prausnitzii* count in this group compared to healthy controls (Lu et al., 2019). The findings from this last study differ from other studies performed in subjects with BD in more advanced stages of their illness, raising the question whether gut microbiota composition might differ according to illness stage or under the influence of pharmacological treatment or physical comorbidities.

Regarding gut microbiota diversity, findings are still contradictory. While some studies did not find any differences in microbiota diversity between individuals with BD and healthy controls (Aizawa et al., 2018; Coello et al., 2019), others found a lower diversity among subjects with BD (Bengesser et al., 2019; McIntyre et al., 2019). Only one study found increased microbiota diversity, especially among patients in a manic episode (Guo et al., 2018). In addition, some studies suggest that alterations in microbiota diversity might be related to mood state (Bengesser et al., 2019; Guo et al., 2018) or illness duration (Painold et al., 2019). Studies focusing on gut microbiota composition of individuals at high risk for BD did not find significant differences in gut bacterial constituents or diversity between unaffected first-degree relatives and healthy controls (Coello et al., 2019; Vinberg et al., 2019).

21.3.2 Gut microbiota as a mood state biomarker for bipolar disorder

Few studies have evaluated differences in microbiota composition according to mood state. On one hand, Painold et al. (2019) found that the Enterobacteriaceae family was more abundant in BD patients with moderate depression, whereas the Clostridiaceae family and *Roseburia* genus were more abundant among those patients already recovered from depression or experiencing mild symptoms. On the other hand, Guo et al. (2018) reported a higher relative abundance of *Escherichia coli* and *Bifidobacterium adolescentis* in manic individuals, whereas depressed individuals showed a higher relative abundance of *Stercoris*. Future studies specifically designed to evaluate changes in microbiota composition across the different mood states will shed more light on this issue (Manchia et al., 2019).

21.3.3 Gut microbiota as a differential diagnosis biomarker for bipolar disorder

So far, only two studies (Rhee et al., 2020; Rong et al., 2019) have reported differences between BD and MDD. In the first study, using stool samples, Rong et al. (2019) found the abundance of Fusobacteriaceae family, *Escherichia blattae* and *Klebsiella oxytoca* to be significantly increased in individuals with BD compared to individuals with MDD, whereas the *Bifidobacterium longum subsp*. and Prevotellaceae family were significantly

reduced. In the second study of Rhee et al. (2020), authors found that *Prevotella 2* genera was more prevalent in the MDD group than in the BD group, through determinations of the composition of serum microbiome by the isolation of bacterial DNA from bacteria-derived extracellular vesicles in the serum. They also found a significantly higher prevalence of the *Ruminococcaceae UCG-002* genera among subjects with MDD. Neither studies found significant differences in alpha diversity between subjects with BD and MDD.

Variations in gut microbiota composition according to different BD subtypes have been described in a pilot study by McIntyre et al. (2019). Although the results are limited by the small sample size, this study reported a higher abundance of *Collinsella* genus among subjects diagnosed with BD type II compared to subjects diagnosed with BD type I. Hu et al. (2019) have also described differences in gut microbiota taxonomic compositions between the two bipolar subgroups. In this study, *Ruminococcus* genus was enriched in BD type II patients, albeit the small number of patients in both groups is again a caveat from the study, which was not originally designed to explore these differences.

21.3.4 Gut microbiota as a biomarker of treatment response in bipolar disorder

Preclinical studies suggest that psychotropic medications can alter gut microbiota composition and/or diversity, which might contribute to the individual variability in treatment response and adverse effects (Maier et al., 2018). For instance, lithium, valproate, and aripiprazole have shown to increase bacterial richness in animal studies (Cussotto et al., 2019). Olanzapine seems to increase the levels of Firmicutes phylum and decrease the levels of Bacteroidetes and Proteobacteria phyla in mice (Davey et al., 2012), although these findings have not been replicated in human studies focusing on patients with schizophrenia (Pełka-Wysiecka et al., 2019). Regarding individuals with BD, Flowers et al. (Flowers, Evans, Ward, McInnis, & Ellingrod, 2017; Flowers et al., 2019) reported that atypical antipsychotics might reduce gut microbiota biodiversity, although these changes were observed only among female participants. Two other studies including drug-free patients with BD found changes in microbial composition after 4 weeks of treatment with quetiapine (Hu et al., 2019; Lu et al., 2019). Hu et al. (2019) described an abundance of *Klebsiella* and *Veillonella* genus in treated patients and lower levels of butyrate-producing bacteria in untreated patients. In a more recent study, Lu et al. (2019) showed an increase of the anaerobic *Bifidobacteria* and *Eubacterium rectale* during the 1-month treatment with quetiapine, although the results from this study are limited by the small sample size and the lack of a placebo group. Altogether, despite gut microbiota seems to be altered following treatment with psychotropics, the available

evidence does not allow to determine if gut microbiota could be useful as a marker of treatment response.

21.4 Main limitations of current research on microbiota-gut-brain axis and bipolar disorder

There are some methodological issues to be concerned about when considering the available evidence on gut microbiota and BD. A major drawback of current works is the limited sample size and the frequent cross-sectional nature of the studies. Given the high variability among individuals, results from studies not including hundreds of people should be interpreted with extreme caution (Allaband et al., 2019). When considering study design, also, it is well known that gut microbiota might be influenced by several factors, such as medication, gender, diet or tobacco use/smoking, which are not always reported in current studies (Goodrich et al., 2014). As so, future studies need to carefully take this confounding factors into account when designing the study, analyzing its data, and drawing conclusions (Allaband et al., 2019; Goodrich et al., 2014).

Furthermore, the composition of gut microbiota varies along the gastrointestinal tract, with a transitional zone in the terminal ileum where the predominant species in the microbiota change from aerobes to anaerobes (Aguirre de Cárcer et al., 2011; Rinninella et al., 2019). This spatial disparity in the gut microbiota should be taken into account when interpreting the results of the studies, since most studies of the human gut microbiota use fecal samples and this may overrepresent certain bacterial populations (Allaband et al., 2019). Likewise, other microorganisms in human gut-like fungi or viruses might also influence human health directly or interacting with gut microbiota (van Tilburg Bernardes et al., 2020), but are rarely analyzed (Allaband et al., 2019; Clavel et al., 2016).

Finally, current measurement tools offer a descriptive information on the taxonomic or genetic composition of gut microbiota, but it does not reflect functional changes. Although still challenging, the analysis of molecules other than DNA (e.g., messenger RNA) or the use of other molecular techniques such as mass spectrometry for measurement of protein (metaproteomics) or metabolite expression (metabolomics) may be more appropriate for that purpose (Allaband et al., 2019; Clavel et al., 2016). Accuracy and reproducibility are other major problems of current studies, yet there are some initiatives for achieving quality standards in this field of research (http://www.microbiome-standards.org) (Clavel et al., 2016).

21.5 Conclusions and future directions

The knowledge on the influence of gut microbiota in mental health is rapidly expanding. So far researchers have hypothesized that alterations in the

MGBA may be involved in the development of an immune response which can finally impact on mental diseases (Fung, 2020). However, many questions about the real role of gut microbiota in mental health remain unanswered, as whether these changes are the cause or the consequence of mental disorders, or how psychotropic medications and health habits influence gut microbiota. More information on the relationship between gut microbiota and psychiatric medications, mood status, neurocognition, or medical comorbidities is needed before alterations in gut microbiota composition can be really considered as potential biomarkers of BD. Moreover, it is important to bear in mind that some alterations in gut microbiota are common to diverse neuropsychiatric disorders, which raises some concern on the specificity of the findings. Likewise, most current data arise from cross-sectional studies, limiting the ability to assess the predictive power of microbiota alterations.

Future studies would need to overcome those caveats. Evaluation of gender differences should be also paramount, considering the probable influence of gender on microbiota composition (Jaggar et al., 2020). In addition, it would be interesting to gain more insight on how gut microbiota alterations correlate with other blood-based or neuroimaging biomarkers (Horne & Foster, 2018). Future studies might also consider exploring alterations in the oropharynx microbiota as it has been proposed that oropharyngeal microbiota, in the same way as gut microbiota, can induce an immune system response (Clark et al., 2013).

A better understanding on the MGBA and its implications on BD would provide a greater comprehension about the etiopathogenics underlying this disorder, while potentially offering the possibility to incorporate biomarkers in the management of these illnesses. But it would also expand the available therapeutic resources. For instance, new treatment approaches for affective symptoms such as augmentation therapies with probiotics or antibiotics, dietary interventions or fecal transplantations are being examined (Dickerson et al., 2018; Jahangard, Yasrebifar, Haghighi, Ranjbar, & Mehrpooya, 2019; Kurokawa et al., 2018; Reininghaus et al., 2018; NCT03279224). Since this area of research is rapidly evolving, further insight on the relationship between alterations in MGBA and BD is expected in the near future.

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