

**INVESTIGATING THE PHYSIOLOGICAL EFFECTS OF GUT THERAPEUTICS IN A
PSYCHIATRIC POPULATION**

by

Evan Forth

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DEDICATION

I couldn't have completed this degree without the support I have received from so many people over the course of my academic journey, and there will never be enough space for me to fully express the gratitude I have for them. None of this would be possible without the many people in my life that chose to support and encourage me in the pursuit of my goals.

I am so incredibly grateful and privileged to have a family that prioritised my education, celebrated with me during my successes, and lifted me up during my failures. The love and support they have shown me throughout my life and academic studies have been the foundation and safety net I needed to strive towards the next challenge. I will always remember the sacrifices they made to invest in my future and never forget how fortunate I am to have such a loving family.

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ABSTRACT

Background: There is a growing body of literature investigating the psychiatric applications of gut microbiome therapeutics. A comprehensive characterisation of this field and novel analyses of physiological changes associated with microbiome manipulation in psychiatry is helpful for identifying the consensus and limitations of the field.

Objectives: 1) Characterise the current landscape of the field of gut microbiome manipulation in psychiatry; 2) Characterise the current understanding of the mechanisms of action for gut microbiome therapeutics, and their physiological effects in the context of psychiatry; 3) Investigate the neurophysiological and microbial effects of probiotics in a psychiatric population.

Methods: To accomplish the aforementioned objectives, numerous narrative and systemic reviews were conducted to evaluate the clinical, physiological, and neurobiological effects of probiotics and fecal microbiota transplantation in psychiatry. Microbial and functional neuroimaging data from the CAN-BIND 12: Effects of Probiotics on Symptoms of Depression clinical trial was then analysed, marking the first analysis of resting state functional connectivity changes associated with probiotic monotherapy to be conducted in individuals with major depressive disorder.

Results: The findings from the reviews suggest probiotics may be effective in treating depressive disorders and could have some anxiolytic properties but may be most effective when administered as an adjuvant treatment. Fecal microbiota transplantation may have a greater immediate effect on psychiatric symptoms, but these effects may be transient in nature. Probiotic intervention has been associated with certain neurobiological changes, and these changes could play a role in ameliorating psychiatric symptoms, but the analysis in chapter 5 found the probiotic intervention to not be associated with resting state functional connectivity changes

traditionally associated with antidepressant response or depression symptom improvement, and to have a generally beneficial effect on the alpha diversity of the gut microbiota.

Conclusions: While there is a consensus in the field on the potential to effect psychiatric symptoms through manipulation of the gut microbiome, the evidence is mixed regarding the clinical effectiveness of gut microbiome therapeutics for treating psychiatric illnesses, and the findings from the primary analysis in chapter 5 suggests they may not affect positive change through altering resting state functional connectivity.

CO-AUTHORSHIP

Many of the chapters in this thesis have been published or submitted to peer-reviewed journals and are a result of collaborative work with my peers and mentors. **Chapter 2** was co-authored with my supervisor Dr. Roumen Milev, fellow graduate students Cassandra Sgarbossa and Arthi Chinna-Meyyappan, and undergraduate students Benjamin Buehner and Ana Storer. **Chapter 3** was co-authored with Dr. Milev, and graduate students Arthi Chinna-Meyyappan and Caroline Wallace. **Chapter 4** was co-authored with Dr. Milev, fellow graduate students Cassandra Sgarbossa and Scott Squires, and undergraduate students Ashley Groth, Maria Farid, Katherine Gallant, Dharmayu Desai, and William Redfearn.

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LIST OF ABBREVIATIONS

AAC	Anterior Cingulate Cortex
AAL	Automated Anatomical Labelling Atlas
ADOS-2	Autism Diagnostic Observation Schedule – Second Version
AFNI	Analysis of Functional NeuroImages
ASD	Autism Spectrum Disorder
ANS	Autonomic Nervous System
BDI	Beck Depression Inventory
BDI-II-R	Beck Depression Inventory (2e) – Revised
BDNF	Brain-Derived Neurotropic Factor
BET	Brain Extraction Tool
BMI	Body Mass Index
BOLD	Blood Oxygen Level Dependent
BSI	Brief Symptom Inventory
CAN-BIND	Canadian Biomarker Integration Network in Depression
CARS	Childhood Autism Rating Scale
CBCL	Child Behavior Checklist
CBF	Cerebral Blood Flow
CBN12: EPSD	CAN-BIND 12: Effects of Probiotics on Symptoms of Depression
CD	Clostridium Difficile
CDI	McArthur-Bates Communicative Development Inventories
CFU	Colony Forming Units
CNS	Central Nervous System

COVID	Coronavirus
CPSS	Cohen Perceived Stress Scale
CRP	C-Reactive Protein
CSC	Chronic Subordinate Colony
CT	Computerized Tomography
CUMS	Chronic Unpredictable Mild Stress
Cz	Midline Central
DBH	Dopamine β -Hydroxylase
DMN	Default Mode Network
DNA	Deoxyribonucleic Acid
DOW	Diary of Workload
DTI	Diffusion tensor imaging
EC	Enterochromaffin
EEG	Electroencephalography
EPI	Echo-Planar Imaging
EPQ-N-12	Eysenck Personality Questionnaire-Neuroticism
EQ-5D-5L	EuroQol 5-Dimension 5-Level Health-related Quality of Life Index
FA	Fractional Anisotropy
FEAT	FMRI Expert Analysis Tool
FMT	Fecal Microbiota Transplantation
fMRI	Functional Magnetic Resonance Imaging
FMRIB	Functional Magnetic Resonance Imaging of the Brain
FRL	Flinders Resistant Line

FSL	FMRIB Software Library
FSL	Flinders Sensitive Line
Fz	Midline Frontal
GABA	Gamma-Aminobutyric Acid
GAD	Generalised Anxiety Disorder
GBA	Gut-Brain Axis
GF	Germ Free
GI	Gastrointestinal tract
GLP	Glucagon-Like Protein
GMDS-R	Griffiths Mental Development Scales - Extended Revised
GSH	Glutathione
GSI	Gastrointestinal Severity Index
HADS	Hospital Anxiety and Depression Scale
HADS-A	Hospital Anxiety and Depression Scale, Anxiety Sub-scale
HADS-D	Hospital Anxiety and Depression Scale, Depressive Sub-scale
HAM-A	Hamilton Anxiety Rating Scale
HAM-D	Hamilton Depression Rating Scale
HOMA-IR	Homeostatic Model Assessment for Insulin Resistance
HPA	Hypothalamic-Pituitary-Adrenal
HT	Hydroxytryptamine
IBD	Irritable Bowel Disease
IBS	Irritable Bowel Syndrome

ICA-ARMOA	Independent Component Analysis based strategy for the Automatic Removal of Motion Artifacts
IL	Interleukin
KSD	Karolinska Sleep Diary
LEIDS-R	Leiden Index of Depression Sensitivity - Revised
M	Mean
MATLAB	MATrix LABoratory
MCFLIRT	Linear Image Registration Tool for Motion Correction
MDA	Malondialdehyde
MDD	Major Depressive Disorder
MET-2	Microbial Ecosystem Therapeutics-2
MNI	Montreal Neurological Institute
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
NLPR-3	NOD-, LRR- and Pyrin Domain-containing Protein 3
NO	Nitric Oxide
OCD	Obsessive Compulsive Disorder
OFC	Orbitofrontal Cortex
OTU	Observed Taxonomic Units
PANSS	Positive And Negative Symptoms Scale
PET	Positron Emission Tomography
PD	Phylogenetic Diversity
PFC	Prefrontal Cortex

PHQ-9	Patient Health Questionnaire
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PSS	Perceived Stress Scale
PTSD	Posttraumatic Stress Disorder
QIDS	Quick Inventory of Depressive Symptomatology
QIIME-2	Quantitative Insights Into Microbial Ecology 2
QoL	Quality of Life
qPCR	Quantitative Polymerase Chain Reaction
RBS-R	Repetitive Behavior Scale - Revised
RCT	Randomized Controlled Trial
RFP	Raw field Potential
RM-ANOVA	Repeated-measures Analysis of Variances
RoB	Risk of Bias
ROI	Regions of Interest
rRNA	Ribosomal Ribonucleic Acid
rsFC	Resting State Functional Connectivity
RSQ	Response Styles Questionnaire
S	Svedberg Unit
SAI	State Anxiety Inventory
SCFA	Short Chain Fatty Acids
SCI	Spinal Cord Injury
SCL-90	Symptom Checklist-90
SCQ	Social Communication Questionnaire

SD	Standard Deviation
SF-36	36-Item Short Form Health Survey
SHC	Non-stressful Single Housing Colony
SNI	Spinal Neuropathic Injury
SNRI	Serotonin-Norepinephrine Reuptake Inhibitors
SPSS	Statistical Package for Social Sciences
SSRI	Selective Serotonin Reuptake Inhibitor
STAI	State-Trait Anxiety Inventory
T	Tesla
TAC	Total Antioxidant Capacity
TAI	Trait Anxiety Inventory
TAU	Treatment as Usual
TH	Tyrosine Hydroxylase
TNF- α	Tumor Necrosis Factor Alpha
TPH	Tryptophan Hydroxylase
UC	Ulcerative Colitis
UF	Uncinate Fasciculus
V	Hypervariable
VABS-II	Vineland Adaptive Behavior Scales-II
VBM	Voxel-based Morphometry
VLMT	Verbal Learning Memory Test

CHAPTER 1

BACKGROUND AND INTRODUCTION

1.1 General Overview

Psychiatric illnesses are highly prevalent, varied, and often difficult to treat conditions that affect the vast majority of individuals worldwide. A study conducted by McGrath et al. estimated that by the age of 75, approximately half the population worldwide can be expected to develop one or more anxiety, mood, substance use, or externalizing disorder (1). When considering that this estimate does not include every psychiatric illness, and the effect psychiatric illnesses can have on family, friends, and community members outside of the individual experiencing the illness, it is likely that everyone at some point in their life will be affected by psychiatric illnesses. Many treatments have been developed for psychiatric illnesses, with pharmacotherapy and psychotherapy being the most common. Though these treatments have been found to be effective in many cases, many barriers can inhibit the treatment of psychiatric illnesses including treatment costs, side effects, specific intolerances, social stigma, and ineffectiveness for some individuals. For this reason, it is important to explore potential alternative treatments, to provide the most options possible to patients, and identify specific treatments that may work best for specific populations. Gut microbiome therapeutics are one such potential alternative treatment for psychiatric illnesses.

The human microbiota has been referred to as the “hidden organ” and consists of the vast community of microorganisms that live within and on the human body and contribute over 150 times more genetic information than the human genome (2). Of this vast community of microorganisms, an estimated 10 – 100 trillion commensal bacteria populate the intestinal lumen,

making up the gut microbiota (3). Together with their genomes, microbial structural elements, metabolites, and environmental conditions, they constitute the gut microbiome (4). The gut microbiome is thought to influence the rest of the body and the brain through the gut-brain-axis (GBA). The GBA is a bi-directional signaling pathway between the gut and the brain with many proposed pathways thought to interact with the digestive, immune, endocrine, metabolic, and nervous systems (5). Research suggests the mechanisms of action for gut microbiome therapeutics, like probiotics, is through the GBA and interactions with the aforementioned systems, but further research is required to completely characterise the specific pathways involved, and their relative contributions to the clinical effects of gut microbiome therapeutics.

The gut microbiome has been found to interact with and modulate the immune system, serotonin production and availability, enteric and central nervous system activity, and the hypothalamic-pituitary-adrenal (HPA) axis, making it a prime target for the treatment of mood and anxiety disorders (6). Research investigating this has mainly focused on three ways to manipulate the gut microbiome: prebiotics, probiotics, and fecal microbiota transplantation (FMT). Prebiotics involve the administration of dietary fibers and specific sugars designed to influence the growth and proliferation of specific bacterial species in the gut microbiome (7). Probiotics involve the ingestion of live bacterial species, usually 1-2 species, that then proliferate in the gut, changing the relative abundance and diversity of bacteria in the gut microbiome (8). Synbiotics combine both probiotics and prebiotics, with the hope that providing the live species as well as the fibers and sugars that they need to grow can result in a more effective product than either alone (9). FMT is a more invasive procedure, designed to repopulate the microbiome of an ill individual through the transplantation of the fecal microbiota from a healthy donor (10). This transplantation can be done through endoscopies, enemas, or the oral consumption of freeze-

dried material. In an area between FMT and probiotics, there have also been studies investigating products that culture many strains of bacteria from a healthy donor and then administer them to the patient (11). This combines the large-scale repopulation technique of FMT with the tolerability and lack of burden of a probiotic.

Therapeutics targeting the gut microbiome to alleviate psychiatric symptoms offer a novel approach that bridges traditional dietary remedies with evidence-based scientific treatment. In many cases, preclinical models have been used to identify specific bacterial strains, metabolites, or substrates with potential ameliorative properties. These preclinical findings can then be translated into gut microbiome therapeutics to be tested clinically. Though many of these therapeutics have a preclinical foundation, their exact mechanisms of action and effects on human physiology have yet to be fully characterised. Developing a full understanding of the physiological effects of gut microbiome therapeutics when used in psychiatric populations is necessary for further improving and innovating said treatments, in addition to identifying predictors of response or populations for which these treatments could be particularly effective. Though research in this field is limited and still has far to go before any definitive conclusions can be made, many clinical trials have evaluated the efficacy of these interventions in alleviating the symptoms of psychiatric disorders, with some studies including the collection of data related to the effects of said interventions on physiological systems and processes.

1.2 State of the Field

The majority of studies in the field have investigated the clinical effects of probiotics on psychiatric symptoms in individuals with depressive disorders, anxiety disorders, or schizophrenia. Some studies have also collected and analysed biomarker data. A biomarker is “a defined characteristic that is measured as an indicator of normal biological processes, pathogenic

processes or responses to an exposure or intervention” (12), and they have been the primary tool for studies wishing to elucidate the mechanisms of action for the observed clinical effects of probiotics and other gut microbiome therapeutics.

1.2.1 Probiotics for the Treatment of Depression

A systematic review conducted by Alli et al., found 19 interventional trials investigating the effect of prebiotics, probiotics, and/or synbiotics on symptoms of major depressive disorder (MDD) as of April 2022 (13). Of these studies, 11 were double-blind, randomized controlled trials (RCT), while the others were open-label (n=4), pilot studies (n=2), or triple-blind RCTs (n=1). Seventeen of the studies were investigating the effects of probiotics, while 3 studies used prebiotics, and 1 used a synbiotic. Ten of the 17 studies on probiotics, 5 of which were RCTs, found a significant decrease in symptoms of depression in the probiotic group. Conversely, the remaining studies found no significant improvements in symptoms of depression in the probiotic groups. The prebiotic studies found no significant improvements, whereas the one synbiotic study observed a significant decrease in depressive symptoms.

These findings suggest mixed evidence in support of the efficacy of probiotics and synbiotics in alleviating the symptoms of depression in individuals with MDD and provide no evidence to suggest prebiotics alone to be effective. The most recently published review was conducted by Asad et al. which identified one additional probiotic trial for MDD, and had similar conclusions as the Alli et al. review (14). These findings are consistent with other systematic reviews investigating the efficacy of prebiotic, probiotic, and synbiotic intervention for alleviating the symptoms of depression. Multiple systematic reviews have found probiotic intervention to have a statistically significant impact on symptoms of depression (15,16), while others have not found their effect to be significant (17).

One possible explanation for the mixed findings from investigations of probiotics for the treatment of depression is the variation in illness severity and progression between study populations. It is possible that gut microbiome therapeutics such as probiotics and synbiotics, are the most effective in treating early stages of depression, or less severe depression, as opposed to depression that is later in its progression or more severe. In addition to studies investigating the efficacy of probiotic and synbiotic monotherapy, some studies have combined microbiome manipulation with traditional antidepressant treatment. These studies suggest the combination of treatments may be more effective than either treatment alone, but these studies are summarised and discussed further in chapter 2 of this thesis.

The field of gut microbiome therapeutics for depressive disorders is still new, so more research will need to be done before a consensus can be reached, but the evidence that is available supports the efficacy of probiotic and antidepressant combination treatment, as well as some evidence to support the efficacy of probiotic monotherapy.

1.2.2 Probiotics for the Treatment of Anxiety Disorders

There unfortunately have not been many clinical studies investigating the effects of probiotics on symptoms of anxiety in populations with anxiety disorders. Some preclinical studies have found certain probiotics to be effective in reducing anxiety-like behaviours in mouse and rat models of anxiety, but most clinical studies have looked at anxiety symptom changes in response to probiotic administration in healthy volunteers, individuals with irritable bowel syndrome, or individuals with depression (17,18). Though these studies were not conducted with participants that were diagnosed with an anxiety disorder, some found probiotic administration to alleviate symptoms of anxiety in these populations.

From a systematic review conducted in 2021, 8 of the studies that were included evaluated the effect of probiotics on symptoms of anxiety, 5 of which reported improvements in anxiety symptoms in response to probiotic intervention (19). These 5 studies were conducted in healthy populations (n=3), or stressed adults (n=2). Of the 3 studies that did not find an effect of probiotics on symptoms of anxiety, 2 were conducted in healthy populations, and 1 had adult participants with moderate mood swings. One on healthy volunteers and the one with participants with moderate mood swings both found probiotic intervention to have no effect on any clinical outcomes (20,21), while the remaining study found an improvement in depressive symptoms but not symptoms of anxiety (22).

With the studies being conducted in populations without anxiety disorders, no conclusions can be made regarding the efficacy of probiotic administration for the treatment of anxiety disorders. That being said, the majority of the studies included in the review, as well as many preclinical studies, have found evidence to support the anxiolytic properties of some probiotics. In addition to this, a study conducted by Eskandrazadeh et al. in 2021 investigated the effects of combined sertraline and probiotic administration for individuals with generalised anxiety disorder (GAD) (23). Their study found a greater statistically significant improvement in the combination group than in the group receiving sertraline monotherapy. These findings suggest that adjuvant probiotic and selective serotonin reuptake inhibitor (SSRI) treatment may be more effective for the treatment of anxiety disorders than SSRIs alone, and that probiotics alone may have some anxiolytic effects, but this has yet to be tested in individuals with anxiety disorders.

1.2.3 Probiotics for the Treatment of Schizophrenia

When investigating the effect of gut microbiome therapeutics on the symptoms of schizophrenia, no studies have investigated the effect of probiotic monotherapy. This could be

due to ethical concerns of providing an investigational product with little evidence to support its efficacy to individuals that may be experiencing acute episodes of psychosis, or it could be that this application is too new to have had large clinical trials conducted. Though probiotic monotherapy for schizophrenia has not been investigated, three large scale, randomized controlled trials have been conducted investigating the effect of adjuvant probiotic administration in combination with antipsychotic medication (24–26). Two of these studies did not find probiotic supplementation to improve measures of psychiatric symptoms, but they did find adjuvant probiotic administration to improve the tolerability and reduce the gastrointestinal and metabolic side effects associated with antipsychotic treatment. Dickerson et al. found that the probiotic group reported fewer bowel problems associated with the treatment than the antipsychotic monotherapy group (24), while Yang et al. found a reduced weight change and body mass index (BMI) in the probiotic group for the first four weeks of treatment when compared to the olanzapine monotherapy group (25). The difference observed by Yang et al. was transient, with no differences observed between the groups after week 4. The third study, conducted by Ghaderi et al., investigated the effects of chlorpromazine and anticholinergic agents with adjuvant probiotics combined with vitamin D compared to adjuvant probiotics alone on symptoms of schizophrenia (26). They found vitamin D and probiotic co-supplementation to be associated with significant improvement generally and improved scores on the Positive and Negative Syndrome scale (PANSS). These findings suggest that probiotic administration alone may not be effective in improving the symptoms of schizophrenia, but may improve the tolerability of antipsychotics, and that probiotics combined with vitamin D may be effective in alleviating the symptoms of schizophrenia or improving the efficacy of antipsychotic medications. One study conducted by Kao et al., also investigated the effect of a prebiotic

supplementation on medicated individuals with psychosis (27). They found prebiotic administration to be associated with improvements in cognition, but to have no effect on BMI or weight gain. These findings suggest the prebiotic administration may be beneficial for the management of psychosis, but far more research will need to be conducted before any conclusions can be drawn.

1.2.4 Biomarker Studies

As would be expected with the novel nature of this field of research, relatively few studies have assessed physiological changes associated with gut microbial interventions in psychiatric populations. The studies that have assessed this have focused primarily on the changes in biomarkers associated with the inflammatory and endocrine systems. A systematic review and meta-analysis was conducted by Amirani et al. in 2020 investigating the effects of probiotic supplementation on biomarkers of inflammation and oxidative stress in patients with psychiatric disorders (28).

The meta-analysis identified studies investigating the inflammatory markers C-reactive protein (CRP) (n=5), tumor necrosis factor-alpha (TNF- α) (n=4), interleukin-1 beta (IL-1 β) (n=3), interleukin-6 (IL-6) (n=3), interleukin-10 (IL-10) (n=3), and nitric oxide (NO) (n=4). Studies assessing oxidative stress biomarkers included glutathione (GSH) (n=5), malondialdehyde (MDA) (n=4), and total antioxidant capacity (TAC) (n=5). The meta-analysis found a significant reduction in CRP, IL-10, and MDA levels associated with probiotic intervention in individuals with psychiatric disorders, a significant increase in GSH levels when studies were stratified by the participants' age, and an increase in TAC in studies with adults and a sample size greater than 50. The observed reduction of CRP and IL-10 suggests probiotic administration may reduce periphery inflammatory signals, which could in turn reduce

inflammation in the central nervous system, and thus influence brain performance, mood, and cognition (29). The observed changes in oxidative stress biomarkers suggests probiotics may play a role in oxidative stress modulation by reducing the damage associated with oxidative balance dysregulation, which has been implicated in the pathogenesis of neuropsychiatric disorders (30).

Besides investigations into inflammatory and oxidative stress biomarkers, some studies have looked at humor and neurohumoral biomarkers. Chong et al. suggested probiotic interventions could beneficially regulate serotonin pathways and stabilize dopamine pathways through observed increases in tryptophan hydroxylase (TPH)-2 and 5-hydroxytryptamine (HT)-6, and reductions in dopamine β -hydroxylase (DBH) and tyrosine hydroxylase (TH) in their probiotic group (31). Probiotic interventions have also been associated with decreased serum insulin levels and homeostatic model assessment for insulin resistance (HOMA-IR) scores (32), and a suppression effect on stress-induced increases in salivary cortisol levels (33). In addition to these studies investigating biomarker changes associated with probiotic use, some studies have investigated the effects of probiotics on neuroanatomical and neurophysiological features. Many of these studies found probiotic use to be correlated with positive neurobiological changes (34), but these studies are reviewed and discussed in detail in chapter 4. The findings from individual studies have not been consistently replicated but have been used to inform the potential mechanisms of action proposed for the effects of gut microbiome therapeutics on psychiatric symptoms.

1.3 Potential Mechanisms of Action

The physiological mechanisms underlying the findings of these clinical studies are not fully characterised, but there are some known pathways that can help explain these clinical and

physiological observations. Nearly all psychiatric illnesses have been found to be linked to the immune system and increased inflammation (35). One way by which gut microbiome therapeutics may exert their therapeutic effect is by decreasing the immune response and inflammation. One potential cause for inflammation is increased intestinal epithelial permeability, also known as “leaky gut syndrome” (36), allowing for pathogens and bacteria from the lumen of the gut to pass into the blood stream resulting in an increased immune response. There is some evidence to suggest gut microbiome therapeutics may reduce intestinal epithelial permeability by lowering serum lipopolysaccharide and zonulin concentrations, helping to alleviate this “leaky gut” and drive a decrease in inflammation (37).

One other pathway that may help explain the effectiveness of probiotics and gut microbiome therapeutics for alleviating the symptoms of depression and anxiety is their interactions with the serotonin system. It is known that approximately 90% of the body’s serotonin is produced in the gut, primarily regulated by enterochromaffin cells (38). Short-chain fatty acids produced by the gut microbiome through the fermentation of non-digestible carbohydrates can interact with metabolite sensors on enterochromaffin cells in the large intestine to increase the production and availability of serotonin. Though peripheral serotonin does not cross the blood-brain barrier, it can still influence the hypothalamic-pituitary-adrenal axis through interactions with corticotropin-releasing hormone and has been linked to anxiety-like behaviour in preclinical models (39).

In addition to the potential effect of microbiome manipulation on serotonin production and availability, certain strains of bacteria have been found to be producers of gamma-aminobutyric acid (GABA) which has special relevance to anxiety disorders as many treatments for acute anxiety are GABA receptor agonists (such as benzodiazepines) (40). Some of the

strains of bacteria reported to produce GABA include strains from the genera *Lactobacillus* and *Bifidobacterium*, both of which are popular genera included in probiotics being investigated for psychiatric use.

As for the effects observed in the schizophrenia studies, the alleviating of gastrointestinal symptoms associated with antipsychotic treatment may be explained through the serotonin pathway as well. In addition to being a neurotransmitter associated with depression and anxiety, serotonin has been found to activate enteric neural circuitry, initiate peristalsis, and reduce constipation (41). Some species of *Bifidobacterium* have also been found to influence weight gain and loss (42), which could explain the reduced weight gain observed in the study conducted by Yang et al.

None of these pathways are fully characterised, and they are certainly not the only ways by which the microbiome may influence the symptoms of psychiatric illnesses, but with further preclinical and clinical research, these mechanisms may become better understood and provide an opportunity to optimize microbiome manipulation for the treatment of illnesses of any kind.

1.4 Limitations of Probiotic Studies

Many of the probiotic and other gut microbiome therapeutic studies have similar limitations that serve as a barrier to drawing conclusions from their findings. The largest limitation is the lack of large scale, double-blind, randomized controlled trials. With this being a relatively new area of research, far more high-quality studies on a variety of psychiatric disorders need to be done to fully determine the clinical relevancy of gut microbiome therapeutics. In addition to the general lack of research, comparing probiotic research is difficult due to the high degree of variability between studies. Probiotic interventions vary greatly in terms of strains

used, dosages, dosing schedule, and mode of delivery. The lack of standardisation and consensus regarding the use of probiotics in research makes it difficult to compare studies. In addition to this, many probiotic and prebiotic formulations are copyrighted by companies, making it impossible to know the specific strains of bacteria used, and forcing researchers to be constrained into using whichever strains a company is willing to provide them. As opposed to traditional antidepressant treatments, like escitalopram, most probiotics studies are using different probiotic formulations with different mechanisms of delivery and dosages. With these limitations in mind, it is clear that probiotics and other gut microbiome targeting therapeutics can be effective in treating the symptoms of some psychiatric illnesses, such as depression. However, it is also just as clear that there is a long way to go before a consensus can be reached and their clinical applications can be determined.

1.5 Conclusions

The studies conducted to date have found mixed evidence supporting the effectiveness of probiotics monotherapy in ameliorating psychiatric symptoms in depressive disorders. The few studies investigating the effects of probiotics on anxiety suggest they may have some anxiolytic properties, but further research is required to determine their effectiveness for treating anxiety disorders. There is no data on the effectiveness of probiotic monotherapy in alleviating schizophrenia symptoms, but some evidence suggests adjuvant probiotics may improve the tolerability of traditional antipsychotic treatments. The benefits of combining probiotics with traditional psychotropic medications have been supported through other adjuvant treatment studies, which are reviewed in chapter 2. Despite the relative lack of studies due to the novel nature of the field, the mixed findings from and significant limitations of the studies that have been conducted, and the lack of clarity surrounding the exact mechanisms of action for gut

microbiome therapeutics, there is a clear connection between the gut microbiome and psychiatry that merits further exploration.

1.6 Rationale

The purpose of this thesis is to summarize the current literature surrounding the use of gut microbiome therapeutics in psychiatry, with a focus on the physiological effects of said therapeutics, and to report the microbial alpha diversity and neurophysiological findings from the Canadian Biomarker Integration Network in Depression (CAN-BIND) 12: Effects of Probiotics on Symptoms of Depression (CBN12: EPSD) clinical trial. Many of the systematic reviews included in this thesis contain information relating to the safety, tolerability, and efficacy of gut therapeutics, as opposed to the physiological changes associated with the treatment, as this has been the focus of the majority of studies conducted to date. The functional neuroimaging analysis presented in Chapter 5 is the first study to examine neurophysiological changes linked to probiotic monotherapy in individuals with depression. This work contributes meaningfully to the understanding of brain-based mechanisms through which probiotics and gut microbiome therapeutics in general may influence mood disorders and can serve as a pilot study and comparison for future neurophysiological analyses.

1.7 Objectives

1. Characterise the current landscape of the field of gut microbiome manipulation in psychiatry
 - a. Evaluate the current literature on the efficacy of probiotics, prebiotics, and synbiotics for psychiatric illnesses.

- b. Systematically review the current literature on the effects of probiotic treatment in conjunction with traditional treatments for psychiatric illnesses.
 - c. Systematically review the current literature on the effects of fecal microbiota transplantation on psychiatric symptoms.
- 2. Characterise the current understanding of the mechanisms of action for gut microbiome therapeutics, and their physiological effects in the context of psychiatry
 - a. Discuss the proposed mechanisms of action for various gut microbiome therapeutics both in isolation and in combination with existing treatments.
 - b. Review the biomarker findings from studies investigating the effects of gut microbiome therapeutics.
 - c. Systematically review the current literature on the neuroanatomical and neurophysiological changes associated with gut microbiome therapeutics and the ways in which probiotic treatments can affect the central nervous system.
- 3. Investigate the neurophysiological and microbial effects of probiotics
 - a. Conduct an analysis of the effects of probiotic administration on brain region functional connectivity in individuals with depression.
 - b. Conduct an analysis of the effects of probiotic administration on gut microbial alpha diversity in individuals with depression.

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CHAPTER 2

SYSTEMATIC REVIEW OF PROBIOTICS AS AN ADJUVANT TREATMENT FOR PSYCHIATRIC DISORDERS

2.1 Prologue

In order to fully characterise the landscape of the field of microbiome manipulation in psychiatry, a review of studies investigating gut microbiome therapeutics used in combination with other psychiatric treatments was necessary. To this aim, a systematic review was conducted using search terms to encompass any clinical trials investigating a combination of gut microbiome therapeutics and psychotropic medication in populations with psychiatric disorders.

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Relative contributions to manuscript: Benjamin Buehner and Ana Storer completed the initial search of the databases, adhering to the search strategy. Evan Forth and one of Benjamin Buehner or Ana Storer independently assessed the titles and abstracts of records retrieved from a systematic search according to the identified inclusion and exclusion criteria. Benjamin Buehner and Evan Forth completed the full-text review. Arthi Chinna-Meyyappan resolved any disagreements. Cassandra Sgarbossa completed quality assessment of eligible articles. All authors contributed to the article and approved the submitted version.

2.3 Manuscript

2.3.1 Abstract

Introduction: Many psychiatric illnesses have been linked to the gut microbiome, with supplements such as probiotics showing some efficacy in alleviating the symptoms of some psychiatric illnesses. The aim of this review is to evaluate the current literature investigating the effects of adjuvant probiotic or synbiotic administration in combination with first-line treatments for psychiatric illnesses. **Methods:** A systematic search of four databases was conducted using key terms related to treatments for psychiatric illnesses, the gut microbiome, and probiotics. All results were then evaluated based on specific eligibility criteria. **Results:** Eight studies met eligibility criteria and were analyzed for reported changes in outcome measures used to assess the symptoms of psychiatric illness and the tolerability of treatment. All Major Depressive Disorder (MDD) ($n = 5$) and Generalized Anxiety Disorder (GAD) ($n = 1$) studies found adjuvant probiotic or synbiotic treatment to be more efficacious in improving the symptoms of psychiatric illness than the first-line treatment alone or with placebo. The schizophrenia studies

($n = 2$) found adjuvant probiotic treatment to have no significant difference in clinical outcomes, but it was found to improve the tolerability of first-line antipsychotics. **Discussion and conclusion:** The findings of the studies included in this review suggest the use of adjuvant probiotic treatment with selective serotonin reuptake inhibitors (SSRIs) for MDD and GAD to be superior to SSRI treatment alone. Probiotic adjuvant treatment with antipsychotics could be beneficial for improving the tolerability of the antipsychotics, but these findings do not suggest that adjuvant probiotic treatment would result in improved clinical outcomes for symptoms of schizophrenia.

Keywords: probiotics, psychiatric illness, psychotropics, adjuvant therapy, gut-brain-axis probiotics, gut-brain-axis, Major Depressive Disorder

2.3.2 Introduction

The human gastrointestinal (GI) tract houses trillions of microorganisms, which have co-evolved with their host and collectively contain over 100 times as many genes as the human genome (Bermon et al., 2015). Colonization of the gut begins at birth, being influenced by the mode of delivery and breastfeeding (Martin et al., 2016) and through the gut's microbial composition somewhat stabilizes throughout adulthood, factors such as the environment, diet, medication, genetics, and age continue to shape microbiota composition and function throughout one's life (Li et al., 2014; Heiman and Greenway, 2016; Odamaki et al., 2016; Cussotto et al., 2019). There is a well-established, bidirectional connection between the gut microbiome and the brain, known as the gut-brain-axis (GBA). This communication includes portions of the sympathetic and the parasympathetic nervous system, the enteric nervous system, as well as both neuroimmune and neuroendocrine signaling (Cryan et al., 2019; Morais et al., 2021; Liu et al.,

2022). Research suggests that the GBA may influence a variety of neurobiological functions, including the pathology of psychiatric disorders.

Major Depressive Disorder (MDD), Generalized Anxiety Disorder (GAD) and Schizophrenia (SZ) are widely known and severe psychiatric disorders. MDD is characterized by pervasive depressed mood and/or loss of interest or pleasure, along with an array of other possible psychiatric and physiological symptoms and is the leading cause of disability worldwide (Evans-Lacko et al., 2018). GAD has a similarly significant impairment in daily functioning (American Psychiatric Association [APA], 2013), and is characterized by a persistent, exaggerated worry about everyday events. MDD and GAD are also somewhat gendered illnesses, with the prevalence in women being reported as 1.5 to 3 times that of men (Vesga-López et al., 2008; Sabic et al., 2021). Antidepressant medications such as selective serotonin reuptake inhibitors (SSRIs) are considered a first-line therapy for MDD and GAD (Gelenberg et al., 2010; Baldwin et al., 2011). SZ is a highly heterogeneous psychotic disorder, characterized by continuous or relapsing episodes of positive symptoms like delusions, hallucinations and irrational thoughts or actions, and negative symptoms like lethargy, apathy, and social withdrawal. Second-generation antipsychotics are considered the first-line treatment for SZ (Patel et al., 2014). Gender differences found in schizophrenia are less consistent, with some reports of equal prevalence between men and women, and some reports of increased prevalence among men (Ochoa et al., 2012).

Various studies have found the microbiota composition of patients with these psychiatric disorders to be significantly different from those of healthy controls (Zhu et al., 2020; Nikolova et al., 2021). Interestingly, fecal microbiota transplants from psychiatric patients to germ-free

rodents have been shown to induce symptoms similar to those associated with the disorders of the donors (Bercik et al., 2011; Neufeld et al., 2011). Certain probiotics or fecal microbiota transplants from healthy patients have also helped alleviate symptoms and induced positive outcomes in patients with psychiatric disorders (Meyyappan et al., 2020; Johnson et al., 2021). As such, there is emerging evidence to suggest that the gut microbiome and the GBA play a crucial role in inducing and modulating psychiatric disorders.

A significant subset of patients affected by these disorders are treatment-resistant or experience adverse effects when taking antidepressants or antipsychotics. Antipsychotic usage is commonly associated with adverse metabolic and endocrine effects such as weight gain and insulin resistance (De Hert et al., 2011), while SSRIs frequently induce unpleasant side effects such as nausea, insomnia, drowsiness and agitation (Hirsch and Birnbaum, 2017). Such adverse effects contribute to low treatment compliance and tolerability. Low compliance and inconsistent efficacy indicate that there is a need to explore alternative treatments or approaches to counteract these unwanted side effects.

Interestingly, both antipsychotics and antidepressants have been found to have antimicrobial properties (Munoz-Bellido et al., 2000; Maier et al., 2018). It is thought that such psychotropic medications can modulate the gut microbiome and consequently influence the GBA. Whether the therapeutic benefits or the adverse effects of these medications are influenced, in part, by their impact on the GBA remains to be determined, however, several recent studies have indicated that the gut microbiome composition could be used as a biomarker to predict pharmacological treatment outcomes (responders versus treatment resistance) in MDD and SZ (Fontana et al., 2020; Ciocan et al., 2021; Yuan et al., 2021). This suggests that the GBA

could play a significant role in the efficacy and tolerability of psychotropic medication. This evidence, combined with the aforementioned therapeutic benefits of microbiome modulation on psychiatric disorders, and the proven ability of probiotics to normalize metabolic issues (Le Barz et al., 2015), suggest that combining psychotropic medication with gut microbiome targeting treatments could have beneficial results. The aim of this systematic review is to evaluate the current literature investigating the effect of adjuvant probiotic or synbiotic (a combination of probiotics and prebiotics) treatment on clinical outcomes and tolerability of first-line psychotropic treatments. We conducted this systematic review as a means to gather scientific evidence and provide a comprehensive and current overview of this topic.

2.3.3 Methods

2.3.3.1 Literature Search Strategy

This review was carried out according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Figure 2-1; Moher et al., 2009). A systematic search was conducted using 4 databases (MEDLINE, EMBASE, PsycINFO, and Web of Science) to identify relevant studies using the following search terms:(antidepressant OR selective serotonin reuptake inhibitors, SSRI OR SNRI OR TCA OR MAOI OR anti-anxiety drugs OR anxiolytics OR benzodiazepines OR beta-blockers OR antipsychotic medication OR mood stabilizer) AND (microbiome OR microbiota OR gut bacteria OR intestinal bacteria OR dysbiosis OR bacteriostatic OR bactericidal OR antibiotic OR bacterial therapy OR bacteriotherapy OR psychobiotic OR microbial therapy OR fecal microbiota transplant OR probiotic) AND (depression OR depressive disorder OR major depression OR bipolar OR mood disorders OR affective disorders OR stress, psychological OR anxiety OR anxiety disorder OR

generalized anxiety disorder OR social anxiety disorder OR PTSD OR OCD OR mania OR panic OR phobia OR psychiatric illness). The database searches were supplemented by retrieval of any additional papers meeting eligibility criteria that were cited in reference lists of relevant review articles yielding 820 additional articles. Searches were conducted in January and February 2022 and yielded 3957 studies after duplicates were removed. Studies that were excluded during full-text screening were rejected due to wrong study design, including the article being a review article or abstract only, and wrong study outcomes. Articles rejected due to “wrong study outcome” did not measure changes in psychiatric symptoms in response to the use of a microbiome-targeted therapeutic as an adjuvant to medication for psychiatric illness.

2.3.3.2 Eligibility Criteria

Eligible articles were restricted to those that were published in peer-reviewed journals and were written in English. Studies eligible for inclusion involved clinical samples that assessed changes in psychiatric wellbeing after standard treatment indicated for psychiatric illness and an adjuvant therapeutic targeting the microbiome.

2.3.3.3 Study selection

Two authors (BB and AS) completed the initial search of the databases, adhering to the search strategy as described above. Two authors (EF and one of BB or AS) independently assessed the titles and abstracts of records retrieved from a systematic search according to the identified inclusion and exclusion criteria. Two authors (BB and EF) completed the full-text review. Any disagreements were resolved by a fourth author (AC). Quality assessment of eligible articles was completed by a fifth author (CS).**Study quality** Quality assessment of articles was completed using Covidence’s built-in, Cochrane Handbook for Systematic Reviews of

Interventions, Risk of Bias (RoB) template. The Cochrane RoB tool assesses the risk of bias for the following domains: sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, and “other” sources of bias. Most studies presented with a low level of bias. Studies where there was no mention of blinding to participants, personnel, outcome assessors, or allocation of treatment, were assigned a “high” judgment in risk of bias. In studies where there was no given description or details regarding one of the RoB domains (i.e., sequence generation), the risk of bias was assigned as “unsure.” A detailed summary of the quality assessment can be found in Table 2-1.

2.3.4 Results

2.3.4.1 Search Results

Following the removal of 1072 duplicates, the search yielded 3,957 results. Subsequent abstract screening and full-text screening, according to the search criteria highlighted earlier (shown in Figure 2-1) resulted in 8 papers with direct relevance to the research question.

2.3.4.2 Study characteristics

Our findings can be grouped into studies examining three major categories of psychological disorders. For one, 370 patients across 5 trials were categorized and treated as patients with MDD (Ghorbani et al., 2018; Miyaoka et al., 2018; Kazemi et al., 2019; Rudzki et al., 2019; Arifdjanova et al., 2021). A significant majority of these patients received traditional antidepressant medication in the form of SSRIs. The sole trial examining 48 patients with GAD (Eskandarzadeh et al., 2021) assessed the use of sertraline, a common SSRI, as its psychotropic agent. The remaining two trials examined 132 patients with SZ or schizoaffective disorder (SZA) (Dickerson et al., 2014; Yang et al., 2021), one in which participants received the atypical

antipsychotic Olanzapine, and the other in which participants continued taking whichever antipsychotic they were prescribed prior to enrolling in the study. As can be seen in Table 2-2, the studies were conducted across 6 different countries and on predominantly female populations. Furthermore, while the majority of studies used an SSRI or atypical antipsychotic, the makeup and the quantity of probiotic administered varied greatly across trials, reducing the generalizability of conclusions.

In all studies, a subset of patients received their psychotropic medication in conjunction with a type of probiotic supplementation. Most of these studies were conducted in the form of a double-blinded, randomized clinical trial. Two studies, however, (Miyaoka et al., 2018; Yang et al., 2021) lacked a placebo arm, following an open-label randomized format.

2.3.4.3 Efficacy of probiotics as an adjuvant therapy

In the eight studies examined (shown in Table 2-2), the medications to treat psychiatric illness were either antidepressants ($n = 6$), or antipsychotics ($n = 2$). All studies included measures for clinical outcomes and symptom severity. In the depression studies, the majority used the Hamilton Depression Rating Scale (Hamilton, 1960) (HAM-D) ($n = 4$) or the Beck Depression Inventory (Beck et al., 1996) (BDI) ($n = 2$) to measure symptoms of mood, anhedonia, sleep, anxiety, appetite, and other symptoms associated with depression. The anxiety study used the Hamilton Anxiety Rating Scale (Hamilton, 1959) (HAM-A) ($n = 1$) to assess symptoms of anxiety such as mood, tension, insomnia, physiological symptoms. The schizophrenia studies used the Positive and Negative Syndrome Scale (Kay et al., 1987) (PANSS) ($n = 2$) to assess positive and negative symptoms associated with schizophrenia such as delusions, hallucinations, blunted affect, and social withdrawal. Higher scores on these scales correspond to an increased severity of the illness.

When examining the effects on symptom severity in patients with MDD or GAD, five of the six studies found that patients who received adjuvant probiotic treatment had significant reductions in symptom severity on the majority of the scales used. One study (Rudzki et al., 2019) did not find any significant effects on symptom severity in patients with MDD receiving adjuvant probiotic therapy using the HAM-D, Symptom Checklist-90 (Derogatis and Savitz, 1999) (SCL-90), and Perceived Stress Scale-10 (Cohen et al., 1983) (PSS-10), but did find that adjuvant probiotic treatment was correlated with increased cognitive performance. Neither of the two studies using patients with schizophrenia or schizoaffective disorder taking antipsychotics found adjuvant probiotic treatment to have any effect on the psychiatric symptoms. That being said, both studies found adjuvant probiotic treatment to reduce the adverse events and side effects associated with antipsychotic treatment, with Dickerson et al. finding fewer reports of bowel difficulties, and Yang et al. finding a reduced weight gain in the first 4 weeks in the adjuvant probiotic groups. Though the reduced weight gain in the study conducted by Yang et al. was transient, with no differences between the adjuvant and monotherapy groups by weeks 8 and 12, adjuvant probiotic therapy was found to eliminate the observed sex-based differences in weight gain seen in the olanzapine monotherapy group. There was no significant difference in body weight change between men and women in the adjuvant probiotic group, whereas there were significantly higher increases in the body weight of women compared to men in the olanzapine monotherapy group.

2.3.5 Discussion

The clinical outcome findings from the studies included in this review suggest probiotic and synbiotic adjuvant treatment with SSRIs for MDD and GAD to be more effective in decreasing depressive and anxious symptomology, respectively, than SSRI treatment alone. In

the one study included in this review that used included a prebiotic group, prebiotics alone were not found to have a significant effect on clinical symptoms. The improved clinical outcomes of probiotic adjuvant treatment for MDD and GAD were found to be persistent throughout the course of the treatment, but further long-term follow-up assessments would be needed to investigate the persistence of this effect when treatment is discontinued. For individuals with schizophrenia, adjuvant probiotic treatment was not found to be more effective in reducing clinical symptom severity than standard antipsychotic treatment alone. Some potential limitations that could explain this lack of effect on clinical symptoms and outcomes are discussed in the conclusion. Though there was no significance in clinical findings for the schizophrenia groups, adjuvant probiotic treatment was associated with a decrease in treatment associated adverse events and side effects. The findings of these studies suggest adjuvant probiotic treatment to have an alleviative effect on some of the gastrointestinal adverse events associated with antipsychotic treatment, such as weight gain and bowel problems. Some of these beneficial effects were found to be fairly long lasting, as is the case in the trial by Dickerson et al. (2014), but some effects were found to be transient, with Yang et al. finding a reduced weight gain for only the first 4 weeks of adjuvant probiotic treatment (Yang et al., 2021).

As mentioned in the introduction, some antipsychotics and antidepressants have been found to have antibacterial properties, as such, it is important to consider the specific medications used in each study. All MDD and GAD studies used participants taking SSRIs, with Miyoaka et al. including participants taking the Serotonin-Norepinephrine Reuptake Inhibitors (SNRIs) duloxetine ($n = 9$) and milnacipran ($n = 3$) and Kazemi et al. including participants taking the tricyclic antidepressant amitriptyline (n not reported). The SSRIs involved in the studies included escitalopram, citalopram, fluoxetine, fluvoxamine, paroxetine, and sertraline. It

has been found SSRIs vary in the degree to which they inhibit bacterial growth, with sertraline and fluoxetine having the strongest antimicrobial activity, followed by paroxetine and fluvoxamine, and then escitalopram and citalopram (McGovern et al., 2019). Amitriptyline has also been found to have antimicrobial effects to around the same degree as paroxetine (Mandal et al., 2010). The antimicrobial effect of SNRIs is less clear, with studies finding venlafaxine to have no effect (Ait Chait et al., 2020), and others finding the clinical effects of duloxetine to be reduced by the bacteria *Ruminococcus flavefaciens* (Lukić et al., 2019), suggesting an interaction between duloxetine and the bacteria. Despite the differences in medications used and their degree of antimicrobial activity, all the MDD and GAD studies found results suggesting probiotics combined with antidepressants improved clinical outcomes when compared to antidepressants alone.

For the schizophrenia studies, all participants in the study conducted by Yang et al. took olanzapine, whereas participants in the Dickerson et al. study took a variety of antipsychotics. The antipsychotics used in the Dickerson et al. study were clozapine ($n = 17$), olanzapine ($n = 15$), risperidone ($n = 15$), aripiprazole ($n = 11$), quetiapine ($n = 9$), haloperidol ($n = 7$), ziprasidone ($n = 5$), and asenapine ($n = 1$), including some participants that took more than one antipsychotic. Though a variety of antipsychotics were used in the Dickerson et al. study, all fall under the category of atypical antipsychotics except for haloperidol which is a butyrophenone derivative. Olanzapine has been found to shift the fecal microbiota in mice toward an “obesogenic” profile (Morgan et al., 2014), and all of the atypical antipsychotics used in the Dickerson et al. study have been found to be associated with significant changes in the gut microbiome and a decrease in species diversity in females (Flowers et al., 2017). Haloperidol has also been found to have some bacteria-inhibiting effects (Korbee et al., 2018). Taking that into

consideration, though the two studies used different antipsychotics, they both used primarily atypical antipsychotics, but the exact degree in which each individual antipsychotic affects the gut microbiome is not certain. As such, it is possible that the use of differing antipsychotics could have contributed to the difference in longevity of the observed beneficial effects.

The exact mechanisms of action for these beneficial effects of probiotic adjuvant treatment are not fully understood. Though the exact pathway and importance of the various pathways by which probiotic adjuvant treatment may exert its effect is not known, multiple pathways have been described for ways in which the microbiome affects the brain and central nervous system. Biomarker data collected in the MDD studies suggests that the probiotic adjuvant treatment exerts its therapeutic effect through effects on the immune system, the hypothalamic pituitary adrenal (HPA) axis, and the tryptophan system. Decreased concentrations of immune markers such as interleukin-6, tumor necrosis factor- α , and nitric oxide suggests a decrease in the activity of the immune system in response to probiotic adjuvant treatment. The immune system has long been known to be intimately linked to MDD and depressive symptomology, with an over-active immune system often being observed in individuals with MDD (Leonard, 2010). One proposed mechanism of action for microbiome targeting treatment for MDD is a reduction in immune system activity through a decrease in gut permeability. It is thought that individuals with MDD and/or other illnesses have increased gastrointestinal permeability, allowing for microorganisms and other potentially harmful toxins from the gut to pass into the body, resulting in an increase in inflammation and immune system activity. Repopulation of the gut microbiome and/or the introduction of beneficial bacteria through probiotic treatment is thought to alleviate this increased gut permeability, and thus reduce inflammation and immune system activity, leading to a decrease in depressive symptomology.

Biomarker data from the study conducted by Arifdjanova et al. (2021) also found cortisol levels to be decreased in the probiotic adjuvant treatment group. Cortisol is often associated with stress and is a major indicator HPA-axis activity. Individuals with MDD and/or GAD often are found to have an overactive HPA-axis and increased levels of cortisol. Probiotic and other gut microbiome targeting treatments have been frequently found to have an inhibitory effect on HPA-axis activity and has been associated with decreased cortisol levels. Influencing the HPA-axis and cortisol production and availability seems to be another pathway by which probiotic adjuvant treatment results in improved clinical outcomes. Another potential mechanism for the improved clinical outcome observed in the probiotic adjuvant treatment groups is through effects on the tryptophan system. Kazemi et al. (2019) and Rudzki et al. (2019) found an increased tryptophan/isoleucine ratio and decreased kynurenine (a tryptophan metabolite) concentration in the probiotic treatment groups of their respective studies. Tryptophan is a precursor to serotonin, a neurotransmitter that has long been associated with MDD and GAD. SSRIs exert their therapeutic action by inhibiting serotonin reuptake transporter proteins, leading to an increase in the relative abundance and concentration of serotonin in the brain. Though serotonin is often associated with the brain and psychiatric illnesses, up to 90% of the body's serotonin is produced in the gut. The mechanism by which the gut microbiome affects serotonin production and availability is thought to be through interactions with microbiome metabolites and enterochromaffin (EC) cells in the gut. The microbiome produces long and short chain fatty acids (SCFA) through the fermentation of non-digestible carbohydrates. Long chain fatty acids influence serotonin production indirectly through interactions with glucagon-like protein (GLP)-1 cells leading to increased GLP-1 which interacts with EC cells to increase serotonin production and availability. Short chain fatty acids interact directly with EC cells to increase serotonin

production and availability. In addition to these interactions with EC cells, short chain fatty acids have the ability to cross the gut-blood and blood-brain barriers, and are thought to have an anti-inflammatory effect, thus further influencing the immune system. The exact bacteria involved in these processes are not fully characterized, but some species of *Lactobacillus* and *Bifidobacterium* have been found to produce neurotransmitters such as acetylcholine and gamma-aminobutyric acid, and *Streptococcus*, *Enterococcus*, and *Escherichia* have been found to produce serotonin, dopamine, and epinephrine (Galland, 2014). These biomarker findings suggest that the beneficial clinical outcomes in the adjuvant probiotic groups with MDD and GAD are a result of the adjuvant probiotic treatment affecting multiple if not all of the pathways by which the microbiome and brain are connected.

As for the observed effect of a decrease in gastrointestinal adverse events in the adjuvant probiotic group of the schizophrenia studies, the mechanism of action is largely unclear. The alleviation of gastrointestinal issues, such as constipation, most likely acted through interactions with the serotonin pathway. As described above, probiotic administration may have resulted in an increase in SCFAs produced by the microbiome, which in turn increases serotonin synthesis through EC cells. Serotonin has been found to activate enteric neural circuitry to initiate peristalsis and reduce constipation (Crowell, 2004). The transient effect of decreasing weight gain for the first 4 weeks could be from a variety of factors. *Bifidobacterium* administration has been linked to both weight gain and weight loss depending on the strain (Yin et al., 2010). The exact strain used by Yang et al. was not reported (Miyaoka et al., 2018), but increased bacteria with bile salt hydrolase has been found to prevent weight gain through the deconjugation of bile acids (Joyce et al., 2014). This may have been the case for the Yang et al. study, but the general negative impact on energy by olanzapine as well as its own mechanism of action for weight gain

may have outweighed the preventative action of the probiotic over time. Although it is thought that probiotics may improve clinical outcomes and symptom severity in populations with schizophrenia through interaction with the immune system (Fond et al., 2020), neither of the studies included a robust collection of immune system related biomarkers, and thus the effect of adjuvant probiotic treatment on the immune system of the patients in these studies is unknown.

2.3.6 Conclusion

Although the studies included in this review were generally found to be of high quality with low risk of bias, there were still some limitations to these studies that impact the generalizability and conclusions that could be drawn from their findings. A major limitation is the small number of studies for each psychiatric illness and the lack of studies investigating other psychiatric illnesses. With only eight studies in total, five of which used a population with MDD, it is difficult to draw strong generalizable conclusions about the effect of adjuvant probiotic treatment on GAD and schizophrenia. Additionally, though the studies included had relatively large sample sizes, further larger scale, double blind, randomized controlled trials are required in the future to make any definitive conclusions. Many of the studies included in this review also did not have comprehensive biomarker collection. To be able to elucidate the mechanisms of action of probiotic supplementation as an adjuvant treatment, as well as to evaluate the colonization of the gut by the probiotics administered and changes in key features of the gut such as intestinal permeability, robust and consistent biomarker collection is necessary. This collection would include biomarkers for the immune system, HPA-axis, serotonin system, and the gut microbiome. Another limitation of the studies was the fact that many used a variety of first-line antidepressant or antipsychotic treatments in combination with the probiotics, which is helpful for evaluating adjunctive probiotic treatment in general but does not give strong insight into the

effectiveness of adjunctive probiotic administration with specific antidepressants and antipsychotics. As different psychiatric treatments can have differing effects on the gut microbiome, studies or analyses focusing on one specific intervention could allow for more detail in determining what combination of treatment would be most effective for an individual. These studies are also limited by their length, with the majority being unable to have significant long-term follow-ups to investigate the longevity of the observed effects. Another limitation in this field is the lack of consensus on dosages for probiotics as well as the treatments they are given in combination with. Dose finding studies in the future are needed, in addition to studies investigating the efficacy of adjuvant probiotic treatment at different stages and severity of the illnesses. This is especially relevant for the schizophrenia studies, which included populations with relatively severe clinical symptoms and later stages of the illness. It is possible that in a population with milder symptoms and a more recent onset, adjuvant probiotic therapy could effectively impact clinical outcomes.

Despite these limitations, the findings of these studies suggest the use of adjuvant probiotic treatment with SSRI treatment for MDD and GAD to be superior to SSRI treatment alone. Probiotic adjuvant treatment with antipsychotics could be beneficial for improving the GI issues associated with antipsychotics, but these findings do not suggest that adjuvant probiotic treatment would result in improved clinical outcomes for symptoms of schizophrenia. Though progress in psychiatric research is challenging, these studies have shown that combining probiotic treatment with first line pharmaceutical treatments is promising, and their findings certainly justify continued research in this area. The gut microbiome and the brain are clearly linked, and these studies show that combining treatments that target both areas, respectively, is a viable and efficacious way to combat the symptoms and treat psychiatric illnesses.

2.3.7 References

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Table 2-1. Summary of quality assessment details and judgement for risk of bias of each study.

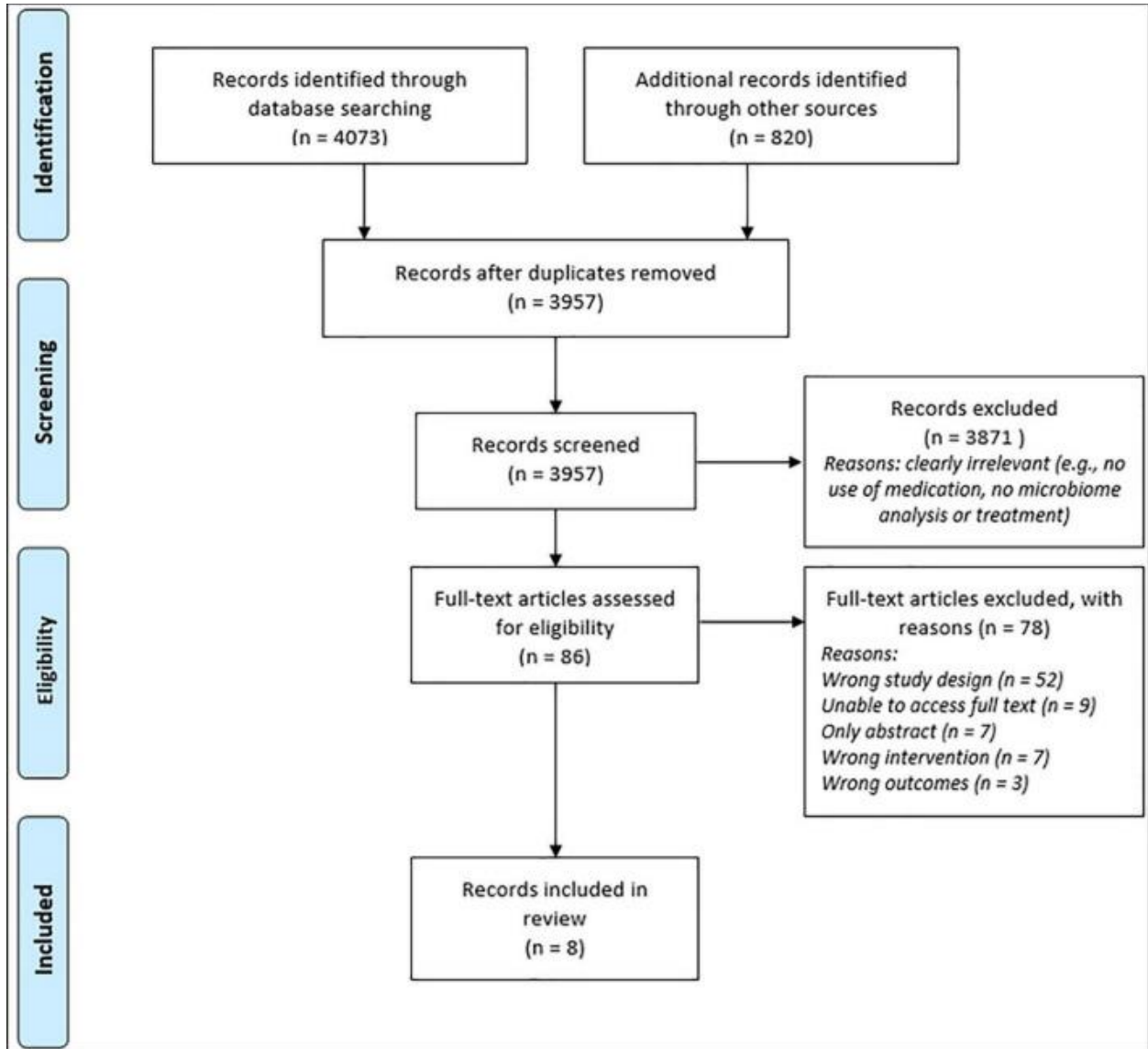
Study	Sequence Generation		Allocation Concealment		Blinding of Participants and Personnel		Blinding of Outcome Assessment		Incomplete Outcome Data	
	Judgement	Comments	Judgement	Comments	Judgement	Comments	Judgement	Comments	Judgement	Comments
Dickerson, 2014	Unsure	No mention of sequence generation.	Unsure	No mention of allocation concealment for placebo vs treatment groups.	Low	Study was double-blind, but there was no mention of how this was maintained.	Low	Study was double-blind, but there was no mention of how this was maintained.	Low	Subjects that were excluded were documented with reasoning.
Kazemi, 2019	Low	Patients were randomly assigned to experimental groups (1:1:1) in blocks of 6.	Low	Participants, clinicians, and raters remained blind to the allocated group of each participant.	Low	Participants, clinicians, and raters remained blind to the allocated group of each participant.	Low	Participants, clinicians, and raters remained blind to the allocated group of each participant.	Low	Subjects that were excluded were documented with reasoning.
Ghorbani, 2018	Unsure	No mention of treatment group sequencing.	Low	Double-blind study with 1:1 ratio for treatment vs placebo randomization.	Low	Double-blind study. Throughout the study, the psychiatrist, the rater (study researchers), and the patients were all blind to allocation.	Low	The raters (study researchers) were blind to allocation.	Low	Subjects that were excluded were documented with reasoning.
Eskandarzadeh, 2021	Low	Patients were randomly assigned using a random numbers table to either treatment or placebo groups.	Low	Patients were randomly assigned using a random numbers table to either treatment or placebo groups.	Low	The study is double-blind, but there was no mention as to how that was maintained.	Low	The study is double-blind, but there was no mention as to if raters were blinded.	Low	Subjects who were excluded were documented with reasoning.
Arifdjavanova, 2021	Low	Participants were randomized to either experimental or control group, but no mention as to how.	High	No concealment of treatment group.	High	No mention of blinding of staff.	High	No mention of blinding of raters.	Low	Some subjects were not included at the beginning, but criteria for which they were not included was not disclosed.
Miyaoaka, 2018	Unsure	No mention of allocation sequence.	Unsure	No mention of allocation concealment.	High	No mention of blinding.	High	No mention of blinding.	Low	All data was reported and subjects that were excluded were documented with reasoning.
Rudzki, 2019	Low	Patients were randomly assigned to placebo or probiotic group using computer generated randomization list.	Low	The study was blinded at group allocator, participant, and assessor levels.	Low	The study was blinded at group allocator, participant, and assessor levels.	Low	The study was blinded at group allocator, participants, and assessor levels.	Low	Subjects that were excluded were documented with reasoning.
Yang, 2021	Unsure	No mention of how the participants were randomized.	High	The blind method was not used in this study, and the researchers were fully aware of the medication.	High	Researchers were fully unblinded and aware of the medication. No mention of unblinding to participants.	High	The blind method was not used in this study, and the researchers were fully aware of the medication.	Low	Subjects that were excluded were documented with reasoning.

Table 2-2. Summary of key study characteristics and outcomes.

Author, year	Study Population	Study design	Sample Size	Mean age (%F)	Country	Intervention Type	Duration	Probiotic	Outcome Measures	Conclusions
<i>Arifljanova et al., 2021</i>	Mild-moderate MDD (ICD-10), 18-45yo	Placebo, double-blind RCT	n = 149	32.9 (62.2%)	Russia	Ciprallex (SSRI) + Placebo or Probiotic	6 weeks	Bac-Set Forte* 3 capsules/day (10 ¹⁰ Colony Forming Units (CFU))	HAM-D for depression severity, ELISA for cortisol and cytokines, HPLC for blood plasma	Found decreased levels of cortisol, dopamine, IL-6, TNF-a and nitric oxide, and a bigger reduction in depressive symptoms in the adjunct PB group compared to standard therapy.
<i>Rudzki et al., 2019</i>	Moderate MDD (DSM-IV-R)	Placebo, double-blind RCT	n = 60	39 (71%)	Poland	Antidepressant (various SSRIs) + Probiotic or Placebo	8 weeks	2 capsules/day (10 x 10 ⁹ CFU of Lactobacillus Plantarum 299v each)	Symptom Severity: HAM-D 17, SCL-90, PSS-10, cognitive function, biochemical parameters also assessed	PB correlated with increased cognitive performance and decreased kynurenine concentration in MDD patients, no significant effect on symptom severity.
<i>Ghorbani et al., 2018</i>	Moderate MDD (DSM-V), 18-55yo	Placebo, double-blind RCT	n = 40	34.8 (70%)	Iran	Fluoxetine (SSRI, 20mg/day - 4W) then Fluoxetine + synbiotic capsule or Placebo (6W)	6 weeks	1 capsule/day (MS probiotic**, 500 mg + prebiotic, 100 mg)	HAM-D primary outcome	Found a greater reduction in HAM-D scores in synbiotic treated patients compared to the placebo group.
<i>Kazemi et al., 2019</i>	Mild-moderate MDD (ICD-10) on medication, 18-50yo	Three-arm placebo, double-blind RCT	n = 81	36.5 (70.9%)	Iran	Antidepressant (sertraline, fluoxetine, citalopram, amitriptyline) + Probiotic or Prebiotic or Placebo	8 weeks	1 sachet/day - probiotic (≥10 × 10 ⁹ CFU Lactobacillus helveticus and Bifidobacterium longum), or prebiotic (galactooligosaccharide)	BDI primary outcome, HPLC for serum tryptophan and branched chain amino acids, ELISA for kynurenine	PB resulted in a decrease in BDI score and increased tryptophan/isoleucine ratio compared to placebo and prebiotic. No significant results for prebiotic and placebo groups
<i>Miyooka et al., 2018</i>	Treatment Resistant MDD (DSM-IV)	Prospective open label randomized	n = 40	43.5, (60%)	Japan	Antidepressant (fluvoxamine, paroxetine, escitalopram, duloxetine, and sertraline) with or without (control) Probiotic	8 weeks	60mg/day (Clostridium butyricum MIYAIRI (CBM588) – 10 CFU/gram)	HAM-D, BDI and the Beck Anxiety Inventory	PB correlated to significant improvement in depression regardless of antidepressant type; well tolerated.
<i>Eskandarzadeh et al., 2021</i>	Drug-free patients with GAD (DSM-V), 18-65yo	Placebo, double-blind RCT	n = 48	33.9 (81.2%)	Iran	Sertraline (SSRI, 25mg/day) + Placebo or Probiotic	8 weeks	1 capsule/day (18*10 ⁹ CFU Bifidobacterium longum, Bifidobacterium bifidum, Bifidobacterium lactis and Lactobacillus acidophilus)	HAM-A scale for anxiety, Beck Anxiety Inventory, State-Trait Anxiety Inventory	Found Sertraline + PB group to have improved clinical outcome measures as opposed to Sertraline + placebo. Significance varied depending on scale used.
<i>Dickerson et al., 2014</i>	Mild-moderate SZ (DSM-IV, PANSS), 18-65yo	Placebo, double-blind RCT	n = 65	46.2 (35.4%)	U.S.	Antipsychotic (various) + Placebo or Probiotic	14 weeks	1 Capsule/day (10 ⁹ CFU combined Lactobacillus rhamnosus strain and Bifidobacterium animalis subsp. lactis strain Bb12)	PANSS to measure psychiatric symptoms + difficulty of bowel movement scale	No significant difference in psychiatric scores, PB well tolerated, PB group had less bowel problems associated w/ treatment.
<i>Yang et al., 2021</i>	First-episode SZ or SZA (DSM-V), 18-55yo	Open-label, RCT	n = 67	43.2 (67.7%)	China	Olanzapine with or without (control) Bifidobacterium group	12 weeks	3 capsules/day (live combined Bifidobacterium, Lactobacillus, and Enterococcus capsules; 1×10 ⁹ CFU each)	Body weight, BMI, appetite, latency to increased appetite, and baseline weight increase of more than 7%, PANSS to measure psychiatric symptoms	No significant differences in PANSS scores. In the first 4 weeks there was reduced weight change and BMI for PB group, but this difference disappeared after 4 weeks. There were no overall differences in appetite.

*Contents of Bac-Set Forte: Streptococcus thermophilus; Bifidobacterium ssp; Lactobacillus ssp. among others. **Contents of MS probiotic: L. casei = 3 × 10⁸, L. acidophilus = 2 × 10⁸, L. bulgaricus = 2 × 10⁹, L. rhamnosus = 3 × 10⁸, B. breve = 2 × 10⁸, B. longum = 1 × 10⁹, S. thermophilus = 3 × 10⁸

Figure 2-1. Flow chart showing literature search and screening process using PRISMA guidelines.



CHAPTER 3

EFFECT OF FECAL MICROBIOTA TRANSPLANT ON SYMPTOMS OF PSYCHIATRIC DISORDERS: A SYSTEMATIC REVIEW

3.1 Prologue

This systematic review serves to expand the scope of gut microbiome therapeutics from the probiotics, prebiotics, and synbiotics discussed in previous chapters to include fecal microbiota transplantation. As a popular method of large-scale gut microbiome manipulation, a systematically reviewing the current literature on fecal microbiota transplantation is a necessary part of characterising the current landscape of the field of gut microbiome manipulation in psychiatry, and the current understanding of the mechanisms of action of gut microbiome therapeutics and their physiological effects in the context of psychiatry.

3.2 Manuscript Information

Status: Published

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Relative contributions to manuscript: All authors have read and approved the manuscript.

Arthi Chinna Meyyappan conceived the idea and hypotheses and designed the systematic review.

Study selection was completed by Arthi Chinna Meyyappan, Caroline Wallace, and Roumen Milev. Arthi Chinna Meyyappan and Evan Forth assessed the data for potential analysis, and wrote a first draft of the text, while all authors contributed to subsequent drafts. Arthi Chinna Meyyappan facilitated article submission.

3.3 Manuscript

3.3.1 Abstract

Background: The Gut-Brain-Axis is a bidirectional signaling pathway between the gastrointestinal (GI) tract and the brain. The hundreds of trillions of microorganisms populating the gastrointestinal tract are thought to modulate this connection, and have far reaching effects on the immune system, central and autonomic nervous systems, and GI functioning. These interactions have also been linked to various psychiatric illnesses such as depression, anxiety, substance abuse, autism spectrum disorder, and eating disorders. It is hypothesized that techniques aimed at strengthening and repopulating the gut microbiome, such as Fecal Microbiota Transplant (FMT), may be useful in the prevention and treatment of psychiatric illnesses. **Methods:** A systematic search of five databases was conducted using key terms related to FMT and psychiatric illnesses. All results were then evaluated based on specific eligibility criteria. **Results:** Twenty-eight studies met the eligibility criteria and were analysed for reported

changes in mood and behavioural measures indicative of psychiatric wellbeing. The studies included were either entirely clinical (n = 8), preclinical with human donors (n = 9), or entirely preclinical (n = 11). All studies found a decrease in depressive and anxiety-like symptoms and behaviours resulting from the transplantation of healthy microbiota. The inverse was also found, with the transmission of depressive and anxiety-like symptoms and behaviours resulting from the transplantation of microbiota from psychiatrically ill donors to healthy recipients. **Conclusion:** There appears to be strong evidence for the treatment and transmission of psychiatric illnesses through FMT. Further research with larger sample sizes and stronger scientific design is warranted to fully determine the efficacy and safety of this potential treatment. Registered on PROSPERO, IRD: CRD42019126795.

3.3.2 Background

In recent years, there has been a growing appreciation for research in the field of the “gut-brain axis” (GBA). The GBA consists of bidirectional biochemical and neural signaling between the gastrointestinal (GI) tract and the brain. Specifically, the gut microbiota can modulate the GBA both directly and indirectly via endocrine, neural, and immune pathways. In disease- or stress-states these pathways may become compromised resulting in intestinal dysbiosis, changes in mood, behavior, and cognition, and altered inflammatory levels ¹.

The gastrointestinal tract is colonized by over one hundred trillion commensal bacteria that exist symbiotically with our bodies. Colonization of the gut occurs at birth and is largely influenced by mode of delivery (cesarean section vs. vaginal birth) and through breast feeding. However, bacterial composition of the gut begins to stabilize throughout adulthood ². Detailed analyses of human gut microbiota have shown considerable individual variability in bacterial

content as this dynamic system is influenced by a variety of factors, such as genetics, diet, metabolism, age, geography, antibiotic treatment, psychotropics and stress ³.

Gut microbiota are critical in the normal development of the immune system, central nervous system (CNS) circuitry, GI functioning, and autonomic nervous system (ANS) functioning. This community of bacteria and its genetic material is often referred to as a virtual organ ³⁻⁵. Studies have since shown that gut bacteria play a vital role in regulating important aspects of brain development and function, along with other host physiology ^{4,6}.

3.3.2.1 The Gut and Psychiatric Symptoms and Disorders

The interaction of the gut with the environmental risk factors of psychiatric illnesses, such as diet and early life stress, suggests that interventions targeting the gut microbiome could prevent and treat psychiatric symptoms ⁷. Psychiatric symptoms can manifest in both psychological and physiological ways, often resulting in impaired functioning. Common physiological symptoms share similarities with symptoms of GI disorders, such as Irritable Bowel Syndrome (IBS). This association may be explained by the close connection between the gut and the brain.

In past studies, individuals with psychiatric illness have also been shown to have a dissimilar microbiota composition compared to healthy individuals, due to decreased diversity and abundance of the healthy gut microbes ⁸. Studies also show that lack of exposure to commensal bacteria, such as in germ-free mice, has significant effects on stress responsiveness in adulthood; it has also been shown that early colonization of the gut with a conventional microbiota, even a single species, can partially reverse these effects ⁹. Some investigations have

shown neurochemical changes as a result of gut microbiome dysfunction, such as altered levels of brain-derived neurotrophic factor (BDNF), reduced serotonin receptor expression, reduced synaptic plasticity gene expression, and increased striatal monoamine turnover^{9–11}.

3.3.2.2 Fecal Microbiota Transplant

Several methods of examining the influence of the gut microbiome on the gut-brain axis have been explored, including manipulating the microbiome via probiotic and antibiotic administration, the use of germ-free animal models, and perhaps most notably, fecal microbiota transplantation (FMT). FMT is the transfer of fecal bacteria from a healthy donor to a recipient. FMT was first used in 4th century China for the treatment of severe food poisoning and diarrhea and other related symptoms¹². However, it is currently only indicated for the treatment of *Clostridioides difficile* (*C. difficile*) infections. *C. difficile* is often contracted by older patients in-hospital following routine pharmacological treatments such as antibiotics. The use of antibiotics often depletes healthy bacteria in the GI tract which can result in microbial dysfunction. FMT is used to restore healthy status of the microbiome via repopulation of healthy bacteria to the gut. Functioning in a similar manner to probiotics, this treatment method helps to maintain the bacterial balance and function. FMT are most commonly accomplished via endoscopies, enemas, and oral feeding of freeze-dried material. Aside from GI and psychiatric disorders, this treatment method is also being explored as a potential treatment for metabolic disorders, autism, multiple sclerosis, and Parkinson's disease^{13–16}. Other variations of this treatment, such as Microbial Ecosystem Therapeutics-2 (MET-2) are also currently being explored, in psychiatric indications such as Generalized Anxiety (GAD) and Major Depressive Disorders (MDD). MET-2 consists of

gut bacteria obtained from stool samples of a healthy donor, chosen for its safety profile, that is then purified and lab-grown prior to being lyophilized and ingested orally by patients ¹⁷.

Two of the most prevalent groups of psychiatric disorders include Major Depressive Disorder and anxiety disorders. MDD is characterized by either depressed mood and/or loss of interest or pleasure, as well other psychiatric and physiological symptoms. Anxiety disorders is a category that includes a variety of disorders characterized by intense feelings of anxiety, nervousness, or fear. These include Generalized Anxiety Disorder, Agoraphobia, Panic Disorder, and specific phobias. Both groups of disorders are characterized by a significant impairment in daily functioning ¹⁸. While there are pharmacological treatments available for both disorders, many people deny treatment due to side effects or stigma-related reasons or are treatment-resistant and unable to find an effective way to improve their symptoms. By targeting the gut, FMT may be a potential way to overcome these drawbacks. Research on the gut-brain axis indicates that there may be a possibility to improve these symptoms through restoration of the gut microbiome via fecal transplant from a healthy donor. However, as this is a relatively novel area of research, there are few studies on FMT in humans as a treatment method in the context of psychiatric disorders.

This review examines findings from preclinical and clinical studies that have examined the effects of endogenous microbiome transfer on psychiatric symptoms. The studies included in this review assess the effects of FMT and related interventions on symptoms associated with a variety of psychiatric illnesses including MDD, anxiety, and chronic stress. Comorbid disorders associated with poor mental health outcomes such as alcoholism and anorexia were also included in several of the studies.

3.3.3 Methods

3.3.3.1 Literature Search Strategy

This review adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Figure 3-1, Moher et al. 2009). Relevant studies were identified by systematically searching the following databases: MEDLINE, EMBASE, CINAHL, PsycINFO, and Web of Science using key search terms including: mood, anxiety, mania, stress, phobia, microbiota transfer, and fecal transplant. The strategy adapted to each of the databases listed above and is described in detail in Appendix A. Searches were conducted in November 2019 and yielded 285 studies after duplicates were removed. The search was updated in April 2020, yielding 7 new studies after full-text screening. Any studies that were excluded during full-text screening were due to wrong study design or outcomes. Reasons for which articles were rejected on the basis of ‘wrong study design’ include the article being a review paper or only an abstract. Those that were rejected due to “wrong study outcome” did not measure any clinical symptoms directly related to psychiatric illness.

3.3.3.2 Eligibility Criteria

All articles eligible for inclusion were published in peer-reviewed journals and were written in English. The studies were restricted to preclinical or clinical samples that were assessed for changes in symptoms of psychiatric illness after undergoing an endogenous microbe transfer via any route of administration.

3.3.3.3 Study Selection

One author (A.C.M) completed initial search of the databases, adhering to the search strategy (Appendix A). Two authors (A.C.M and C.W) independently assessed the titles and abstracts of records retrieved from the systematic search according to the identified inclusion and exclusion criteria and completed the full-text review. Any disagreements were resolved by a third author (R.M).

3.3.3.4 Study Quality

The Cochrane Handbook for Systematic Reviews of Interventions Risk of Bias Tool addresses 6 specific domains in assessing the quality of randomized controlled trials: sequence generation, allocation concealment, blinding, incomplete outcome data, selective outcome reporting and ‘other issues’. All included studies were analyzed according to these domains by A.C.M and C.W. As there is limited research on this topic, studies that did not follow the double-blind, randomized controlled model, were included if they had a comprehensive methodology after thorough analysis by three of the authors (A.C.M., C.W., and R.M.). Overall, the included studies presented with low levels of potential bias save for cases where blinding of either the participant or study team was not performed. For these cases, bias was assessed as high. Detailed assessments can be found in Table 3-1. Allocation concealment was not assessed since no studies concealed treatment allocation from subjects or participants.

3.3.4 Results

3.3.4.1 Study Characteristics

This review contains 28 studies evaluating the effect of fecal microbiota transplants on various psychiatric and physical symptoms. Of these, eleven studies examined exclusively

animal samples, nine studies were preclinical subjects with transplanted microbiota from human donors, and eight studies examined exclusively human samples. The characteristics of these studies are displayed in Tables 3-2, 3-3, and 3-4. The average sample size was n=23 for entirely preclinical studies, and n=78 for preclinical studies with human donors, and n=19 for clinical studies. Four of the preclinical studies failed to report rodent sample size. The symptoms most frequently studied were that of Irritable Bowel Syndrome (IBS), chronic stress, and depressive-like symptoms.

3.3.4.2 Preclinical Studies

In the eleven preclinical studies included in this review (Table 3-2), the primary indications investigated were chronic stress (n=6), alcoholism (n=2), chronic neuropathic pain (n=2), and depression (n=1). Of the studies assessing chronic stress, three investigated the effects of FMT from mice subjected to the chronic unpredictable mild stress (CUMS) procedure to healthy mice, one replicated this using the social defeat model of stress in rats, one investigated the effects of fecal transplant from healthy mice to stressed mice, and one investigated the effects of FMT from NOD-, LRR- and pyrin domain-containing protein 3 (NLPR3) knockout mice on the resiliency of mice subjected to the CUMS procedure after fecal transplant. When investigating the effects of FMT from CUMS mice to germ free mice, three studies found that the FMT resulted in increased anxiety and depression-like behaviour^{19–21}. Mice were assessed using the open-field, tail suspension, forced swimming, and elevated plus maze tests – all standard tests for evaluating symptoms related to stress and depression in the field of depression research in mice. Pearson-Leary et al. conducted a similar study, by using the social defeat rodent model of stress. Male rodents were introduced into cages with retired male breeder rats

that were preselected for aggression. This creates a situation where the experimental rats are subjected to several episodes of social defeat by the older, aggressive rats, leading to chronic stress. These rats were then identified as SL/vulnerable (exhibiting passive behaviours and short-latencies to defeat) or LL/resilient (exhibiting active coping behaviours and long-latencies to defeat). SL/vulnerable rats displayed increased depressive and anxiety-like behaviours, whereas LL/resilient rats did not. Naïve rats then received FMT from SL/vulnerable, LL/resilient, or control rats. It was found that rats receiving FMT from the SL/vulnerable group displayed depressive-like behaviours when assessed with the forced swim test while the LL/resilient group was not significantly different than control²².

Langgartner et al. investigated the alleviating properties of FMT by designing a study in which mice were split into chronic subordinate colony housing (CSC) and non-stressful single housing (SHC). CSC housing is a validated way to subject mice to chronic psychological stress, similar to the CUMS procedure. The researchers then evaluated if frequent FMT from SHC to CSC mice could stop the development of anxiety and depression-like symptoms. It was found that these transplants were mildly stress protective, resulting in decreased anxiety and depression-like symptoms in the recipient mice²³. Zhang et al. also investigated the protective properties of FMT by transplanting the microbiota from NLPR3 knockout mice into GF mice. NLPR3 is a gene involved in the aforementioned immune pathway that modulates the GBA. The transcripts of this gene have been found to be increased in both patients with depression and mouse models for depression^{24,25}. Knocking out this gene is thought to be protective against the development of depressive-like symptoms in mice. These mice were then subjected to the CUMS procedure in order to see the effects of the FMT on their resiliency. It was found that

FMT from NLPR3 knockout mice resulted in a decrease in anxiety and depressive-like behaviours in the recipient mice ²⁶.

Xiao et al. evaluated the transfer of alcohol-withdrawal symptoms via FMT. In this study, the donor mice were treated with alcohol – they were forced to ingest alcohol for two weeks, with the concentration of alcohol increasing from 5% to 35% over that time period. The fecal microbiota of these mice was then transplanted into healthy control mice. This transplantation resulted in depressive behaviour in the recipient mice. This behaviour was evaluated using the forced swim and tail suspension tests ²⁷. Jiang et al. conducted a similar study treating mice with alcohol and transplanting their microbiota into germ free mice, but some alcohol treated mice were treated with nicotinamide riboside afterwards. Similar results were found after FMT from alcohol treated mice, but treating the donors with nicotinamide riboside before transplantation stopped the transmission of depressive-like behaviours ²⁸. Additionally, Tillmann et al. also studied the use of FMT to transfer depressive behaviour, using a Flinders sensitive line (FSL) and Flinders resistant line (FRL) rats. FSL and FRL rats have been widely used for over 30 years as a depression model in rats ²⁹, where FSL rats display depressive-like behaviours, and FRL rats are resistant to the development of depressive-like behaviours. Tillman et al. Investigated the effects of FMT from FSL and FRL rats to FSL, FRL and saline control groups. The only significant behavioural results found were an increased immobility in the forced swim test and decreased time spent in the centre of the open field test resulting from FMT from FSL to control rats, and that rats receiving FRL feces struggled less in the forced swim test than those receiving saline. The former suggests the transfer of depression symptoms from FSL rats to others, and the latter provides evidence against the transference of resiliency from FRL rats. Yang et al.

measured pain and depression symptoms in mice after FMT from rats with neuropathic pain presenting with and without anhedonia. Rats were administered a spinal neuropathic injury (SNI) and then assessed for anhedonia susceptibility. After being separated into anhedonia-susceptible and resilient groups, the rats' microbiota was then transplanted into mice, and the mice were assessed for pain responses and depression-like symptoms. It was found that transplants from anhedonia susceptible rats aggravated pain and depression-like symptoms, and those receiving from anhedonia resilient microbiotas had improved pain and depression-like symptoms. The reverse was conducted by Schmidt et al. 2020, investigating the effects of FMT from healthy rats to rats given a spinal cord injury (SCI). It was found that the transplantation of a healthy microbiota resulted in a reduction of anxiety and depressive-like symptoms in mice subjected to SCI, as measured by the elevated plus maze and light-dark tests.

3.3.4.3 Preclinical Studies with Human Donors

Of the nine preclinical studies performing FMT from human donors to germ free (GF) mice (Table 3-3), four assessed the effects of FMT from patients with depression, one from patients with alcoholism, one from patients with anorexia and one from patients with IBS. Two studies assessed the ameliorating properties with the transfer of gut microbiome from healthy human controls to mouse models of alcoholism and autism spectrum disorder respectively. These mice were then assessed for changes in various tests used to indicate change in psychiatric state.

After FMT from individuals with depression to GF mice, three studies found a decrease in centre motion in the open field test^{30–32}. Two studies also found a significant increase in immobility duration in the tail suspension and forced swim tests^{30,32}. Liu et al. found increase in immobility duration in the forced swim test only³³. Although Kelly et al. did not find any

changes in the forced swim test, they found a decrease in the total visits to the open arms in the elevated plus maze ³¹. These four studies all support that FMT from patients with depression to GF mice can result in depression-like behaviour in the recipient mice.

Alcoholism is highly comorbid with mood and anxiety disorders, with someone with alcoholism being 3.6 and 2.6 times more likely to have a mood or anxiety disorder respectively ³⁴. In the studies investigating the effect of FMT on depressive and anxiety-like behaviors related to alcoholism, Zhao et al. found that transplants from patients with alcoholism to GF mice resulted in depression and anxiety-like behaviours in the aforementioned tests ³⁵. The inverse was conducted by Xu et al. – in their study, mice were treated with alcohol and as a result these mice displayed depression and anxiety-like symptoms in the same tests. FMT was then performed from healthy human donors at different time points throughout the alcohol treatment. Xu et al. found that when administering three FMTs per week, if the transplants began before or during the alcohol treatment, the depression and anxiety-like behaviours were not observed ³⁶. Chen et al. conducted a similar study investigating the positive effects of FMT from healthy humans to mice models for autism spectrum disorder. The mice either received a transplant from the original pooled human gut microbiome, or from cultured bacteria from the original pooled human microbiome. It was found that both the in vitro cultured and non-cultured bacteria transplants resulted in anxiety-like behaviours assessed through the marble burying and self-grooming tests, but that only the non-cultured bacteria transplants resulted improved performance during the open field test.

Anorexia is also closely tied to mood and anxiety disorders, with the likelihood of someone with anorexia having a comorbid mood or anxiety disorder being 2.4 and 1.9 times

higher respectively than the general public³⁷. When assessing the effects of FMT from patients with anorexia to germ free mice, Hata et al. observed an increase in compulsive and anxiety-like behaviours in the mice when assessed using the open field and marble burying test. As previously seen, the mice spent significantly less time in the centre of the open field test, suggesting anxiety-like behaviour. These mice also buried more marbles than control mice, suggesting compulsive behaviour³⁸. These findings are also consistent in De Palma et al.'s study where transfer of anxiety and IBS symptoms to GF mice via FMT was assessed. In their study, it was found that transplants from anxious IBS donors resulted in more anxiety-like behaviour in recipient mice. This was not the same for transplants from IBS donors without anxiety³⁹. These studies all show that FMT can confer certain traits of the donor's psychiatric illnesses to the recipient mouse, and that transplants from healthy donors may be able to alleviate some psychiatric symptom.

3.3.4.4. Clinical Studies

In contrast to the preclinical studies with human donors, where transplants were primarily from ill and healthy humans into GF mice, in clinical studies (Table 3-4), the fecal microbiota of healthy volunteers were transplanted into humans with illnesses such as IBS and depression. All eight clinical studies assessed for psychiatric symptoms – six studies assessed depressive symptoms, four assessed anxiety symptoms, one assessed neuroticism, two assessed quality of life in relation to IBS, and one assessed fatigue. Depression symptoms were assessed in four of the six clinical studies using the Hamilton Depression Rating Scale (HAM-D). Other scales used to assess depression symptoms were the Patient Health Questionnaire (PHQ-9), the Quick Inventory of Depressive Symptomatology (QIDS) and the Hospital Anxiety and Depression

Scale, depressive sub-scale (HADS-D). All of these studies found a significant short-term improvement in depression symptoms. The long-term effects were less consistent, with three studies finding a return to baseline at week 12, week 20, and month 6 respectively ^{40–42}. Xie et al., however, found a persistent decrease in depression symptoms lasting up to 17 months after the final round of FMT ⁴³.

Of the four studies assessing anxiety symptoms, three used the Hamilton Anxiety Rating Scale (HAM-A), and one used the Hospital Anxiety and Depression Scale, anxiety sub-scale (HADS-A). Three of the four studies found a significant improvement in anxiety symptoms following FMT ^{40,42,44}. Although Mizuno et al. found improvement in the anxiety scores as well, it was not significant ⁴². As with depression symptoms, Huang et al. found the anxiety scores to return to baseline within six months post-transplant and Mazzawi et al. found the improvement to be insignificant by week 20 ^{40,41}.

Neuroticism was assessed using the -1 (EPQ-N-12) by Mazzawi et al. ⁴¹. A significant decrease in EPQ-N-12 scores was seen at week 3, but this returned to baseline by week 20 ⁴¹. Huang et al. and Johnsen et al. assessed quality of life using the IBS-QOL scale. Scores on this questionnaire followed a similar pattern as the assessments above, with a significant improvement being observed during the first 3 to 6 months and returning to baseline after 6 to 12 months ^{40,45}. Johnsen et al. also found a similar effect on fatigue, with improvement up to 6 months after FMT and a waning effect from 6 to 12 months ⁴⁵.

3.3.5 Discussion

The findings from reviewing the included studies suggest that FMT can affect symptoms of psychiatric disorders. This was shown for both the relief of psychiatric symptoms resulting from the transfer of microbiota from healthy donors to ill recipients and the transmission of symptoms through the transplantation of microbiota from ill donors to healthy recipients. This relationship was investigated in a variety of psychiatric disorders including depression, anxiety, anorexia and alcoholism. The transmissible properties of FMT were also well demonstrated in these studies. Notably, regardless of donor species, the transmission of psychiatric symptoms from ill donors to GF mice was consistently found. This was supported in multiple studies, with observed transference of symptoms from mouse models of depression, anxiety, chronic stress and alcoholism, and from humans with depression, to GF mice. This provides support for the concept that the gut microbiome may both contribute to the development of psychiatric disorders and be a viable target for treatment for these disorders.

All included clinical studies found improvement in the symptoms of psychiatric disorders related to these disorders following FMT from healthy donors. The beneficial aspect of FMT from healthy donors was also demonstrated preclinically where healthy transplants resulted in alleviation of depression- and anxiety-like symptoms that appeared in mice subjected to certain conditions. This alleviation of symptoms was found in mice experiencing alcohol withdrawal, as well as stressful living conditions. Though symptom alleviation was consistently observed, the duration of improvement was inconsistent. Some studies, such as Xie et al., observed an alleviation of symptoms that seemed to last indefinitely, but the majority found this to be transient^{40–43}. The benefits seemed to last for only around 3-6 months, which, if used as a treatment for psychiatric disorders, is a limitation for FMT in clinical practice.

3.3.5.1 Mechanism of Action

The mechanism of action for how this gut microbiome modulation results in the observed symptomatic changes has yet to be fully understood. There are currently a few major hypotheses for how the microbiome affects the nervous system, resulting in symptomatic changes. The papers included in this study discussed some of these theories, with the majority postulating the mechanism to be through changes in serotonin production, immune response, and metabolism in response to microbiome changes. Serotonin transmission has long been known to be altered in depression, with selective serotonin reuptake inhibitors (SSRIs) being the most prescribed treatment for depression ⁴⁶. An estimated 90% of the body's serotonin is produced by enterochromaffin (EC) cells in the digestive tract ⁴⁷. The functioning of these cells has been known to be affected by gut microbiome changes. One way that microbiome disruption is thought to affect serotonin production is through short chain fatty acids (SCFAs). SCFAs are produced by the gut microbiome through the fermentation of non-digestible carbohydrates, suggesting that treatments that ameliorate gut health can influence SCFA concentrations. SCFAs, particularly butyrate and propionate influence the synthesis of the rate limiting enzyme tryptophan hydroxylase which synthesizes serotonin produced by EC cells ^{48,49}. In addition to their role in serotonin production, SCFAs also can cross gut-blood and blood-brain barriers during immune signaling. The immune system can be affected by the gut simply by the fact that there are many immune cells located in the gastrointestinal tract, meaning that gut disruption can also disrupt these cells. The SCFAs produced by the gut microbiome have anti-inflammatory properties and can work to regulate the immune response ⁵⁰. In the gut, they influence expression of anti-inflammatory markers, such as interleukin (IL-)10 in macrophages and

intestinal dendritic cells⁵¹. In the central nervous system, SCFAs have additional roles, such as regulating maturity and function of microglia (macrophages in the brain that are part of the brain's immune response)⁵². Many psychiatric disorders have been linked to inflammation and an increased immune response, as observed through elevated levels of immune marker cytokines⁵³. It is hypothesized that this response is mediated by the NLRP3 inflammasome, a multiprotein intracellular complex that activates pro-inflammatory cytokines⁵⁴.

A more direct way that the microbiota influences the central nervous system is through interaction with the vagus nerve. The vagus nerve is comprised of 80% afferent nerve fibers and 20% efferent fibers. Afferent nerve fibers of the vagus nerve are affected by metabolites of the microbiota that then take that information back to central nervous system⁵⁵. This is hypothesized to influence central and peripheral changes resulting in alleviation of psychiatric symptoms. More specifically, the vagus nerve is affected by long and short chain fatty acids both directly and indirectly, through cellular production of neurotransmitters, such as serotonin⁵⁶.

These mechanisms may provide insight into the use of FMT as a potential treatment for psychiatric symptoms, such as mood and anxiety. The repopulation of the gut microbiome with healthy bacteria through FMT may have positive neurobiological, immune, and metabolic effects which in turn may influence the trajectory of the psychiatric indication.

3.3.5.2 Treatment Feasibility

Using FMT as a treatment for psychiatric illnesses in the future is an interesting idea that merits exploration. MDD and anxiety disorders affect millions of people worldwide and have a very large burden to the individual and society as a whole. The current gold-standard treatment

for psychiatric illnesses, MDD and anxiety disorders in particular, are antidepressant medications. Though antidepressants have a relatively high efficacy, a large proportion of individuals with psychiatric illnesses do not respond to these first line treatments and thus need to try alternatives⁵⁷. Further, many antidepressant users also experience side effects such as restlessness, nausea, vomiting, anxiety, insomnia, sexual dysfunction, gastrointestinal cramps and diarrhea, and headaches that can make the arduous process of searching for effective treatments even harder⁵⁸. Antidepressant medications are also still steeped in stigma further impeding one's ability to ask for and receive help and treatment. Finally, as it stands on average antidepressants can be costly, especially without insurance or government-funded healthcare.

There is a great need for new therapeutic targets and treatments in order to provide options and better help individuals suffering from these psychiatric illnesses. When considering the findings demonstrated in this review, FMT appears to be a promising candidate for this. The ongoing research certainly suggests its efficacy and given the few side effects and adverse events reported in these papers, may even challenge the treatments currently available. Though the treatment effect seems transient, symptoms appeared to improve relatively quickly after treatment. Another common issue seen in these indications are often to do with treatment adherence. However, given FMT effects can last up to 6 months, it may be easier to adhere to than a daily medication or a weekly psychotherapy appointment. Assuming one transplant is sufficient for therapeutic benefits lasting up to six months, the cost of treatment may be comparable to that of brand-name antidepressants, however not much is known about the costs of FMT⁵⁹. There is potential, however, for cost of FMT to decrease, as treatment becomes more mainstream and modified.

Though the effectiveness and tolerability of FMT, as seen in these studies, makes it a promising potential treatment, there are some aspects that could limit its adoption into mainstream clinical settings. A potential drawback currently is the procedure itself. Although costs are comparable to antidepressants, it is still relatively expensive and a labor-intensive alternative to other psychiatric treatments. Additionally, the safety of FMT has also not been sufficiently understood and its associated stigma is still unknown. These points, along with the treatment still being in the early stages of research, make it difficult to fully determine the feasibility of FMT as a treatment for psychiatric illnesses such as depression and anxiety.

3.3.5.3 Limitations

Although the studies included in this review were of good quality and contributed to a greater understanding of FMT in the context of mental health and illness, there are considerable limitations. A significant limitation in any FMT study is the fact that although research on the gut microbiome has been prolific, we still do not know what a ‘healthy microbiome’ is. Some researchers refer to a healthy microbiome as one of an individual with no overt diseases and others, however, even among those who are considered healthy, the variation in taxonomic composition is great^{60–62}.

The main limitation of the clinical studies were small sample sizes. The lack of large-scale, double blind randomized controlled trials makes it difficult to determine efficacy and safety. The majority of clinical studies also assessed the psychiatric symptoms in individuals with IBS, and not necessarily those exclusively with psychiatric disorders. This means that, though there was clear improvement in psychiatric symptoms, it cannot conclusively be said that FMT will improve the symptoms of individuals with psychiatric disorders. Additionally, it is

possible that the improvement of psychiatric symptoms is secondary to the improvement of gastrointestinal symptoms associated with IBS, thus is not a direct relationship.

For the preclinical studies using human donors, the sex of the mice and donors was a major limitation, given most studies used donors or mice of only male sex. Given that there are clear sex and gender differences in the prevalence and symptomatology of mental illnesses, further research is warranted to determine if sex and gender have an effect on the efficacy of FMT procedures. Some of these studies also included donors that were taking various medications, including antidepressants, which may have affected the results. Additionally, the administration of antibiotics to create GF recipient mice and the variability in FMT administration protocol make the findings difficult to translate. For instance, some recipients received multiple FMTs, while others received only one and the justification for choosing donors varied. The heterogeneity of indications studied also creates difficulty in knowing, with any certainty, how efficacious this procedure will be for a given indication. Without a consensus on a standard procedure for conducting this research, it is difficult to compare results between studies.

3.3.6 Conclusion

With high individual variability in symptomatology and prognosis, high levels of comorbidity with other disorders, genetic *and* environmental influences, progress in research in treatment of psychiatric disorders has been challenging. Given the huge heterogeneity of psychiatric disorders, finding treatment that works for all patients is not achievable, especially given the range of factors that influence the disorder and treatment response. While the research in this field is far from complete, the potential of targeting the gut-brain axis using FMT to alleviate symptoms of psychiatric illness is promising. Additionally, given the adaptable nature

of the gut microbiome, it may be a good representation of the individual's history and could explain differences in risk of illness, disease course, and response to treatment. If these therapies are able to alleviate symptoms of psychiatric disorders, they could be offered to some patients as personalized, alternative, and/or adjunctive treatments to combat specific symptoms that tie together specific gut bacteria strains or the gut, as a whole, to the brain.

3.3.7 References

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Table 3-1. Quality Assessment Details

Study	Blinding of outcome assessors for all outcomes		Blinding of participants and personnel for all outcomes		Incomplete outcome data for all outcomes	Selective outcome reporting	Sequence generation	
	Judgement	Comments	Judgement	Comments	Judgement	Judgement	Judgement	Comments
Cai 2019 [66]	High	Single outcome group, no blinding of any parties involved	High	Single case, no blinding of any parties involved.	Unclear	Unclear	High	Single case, no sequence generation
Tillmann 2019 [32]	Low				Low	Low		
Kurokawa 2018 [48]	High	Outcome assessors not blinded.	High	Participants and personnel not blinded.	Low	Low		
DePalma 2017 [43]	High	Investigators not blinded for behavioural and gastrointestinal motility tests.	Low		Low	Low	Low	
Xiao 2018 [29]	Low		Low		Low	Low	Low	
Mazzawi 2018 [46]	High	Outcomes assessors not blinded.	High	Participants and personnel not blinded.	Low	Low	Low	
Xu 2018 [40]	Low		Low		Low	Low	Unclear	
deClercq 2019 [67]	High		High		Unclear	Unclear	High	
Huang 2019 [36]	Low		Low		Low	Low	Unclear	

Study	Blinding of outcome assessors for all outcomes		Blinding of participants and personnel for all outcomes		Incomplete outcome data for all outcomes	Selective outcome reporting	Sequence generation	
	Judgement	Comments	Judgement	Comments	Judgement	Judgement	Judgement	Comments
Huang 2019 [45]	High	Outcomes assessors not blinded.	High	Participants and personnel not blinded.	Low	Low		
Li 2019 [21]	Unclear		Unclear		Low	Low	Low	
Zhang 2019 [28]	Low		Low		Low	Low	Unclear	
Xie 2019 [47]	High	Outcome assessors not blinded.	High	Participant and personnel not blinded.	Low	Low		
Zhao 2019 [39]	Low		Low		Low	Low	Low	
Kelly 2016 [34]	Unclear		Unclear		Low	Low	Low	
Mizuno 2017 [44]	Low		High	Participants and personnel not blinded.	Low	Low		
Langgartner 2018 [25]	High	Outcome assessors, aside from histological scoring, were not blinded.	High	Personnel not blinded.	Low	Low	Low	
Yang 2019 [68]	Unclear		Unclear		Low	Low	Low	
Hata 2019 [42]	Unclear		Unclear		Low	Low	Unclear	

Study	Blinding of outcome assessors for all outcomes		Blinding of participants and personnel for all outcomes		Incomplete outcome data for all outcomes	Selective outcome reporting	Sequence generation	
	Judgement	Comments	Judgement	Comments	Judgement	Judgement	Judgement	Comments
Lv 2019 [22]	Unclear		Unclear		Low	Low	Low	
Zheng 2016 [35]	Low		Low		Low	Low	Low	
Pearson-Leary 2019 [24]	Low		Low		Low	Low	Low	
Schmidt 2020 [33]	Low		Low		Low	Low	Low	
Johnsen 2020 [49]	Low		Low		Low	Low	Low	
Jiang 2020 [30]	High	Outcome assessors not blinded.	Low		Low	Low	Low	
Chen 2020 [69]	High	Outcome assessors not blinded.	Low		Low	Low	Low	
Siopi 2020 [23]	Low		Low		Low		Low	
Liu 2020 [37]	High	Outcome assessors not blinded.	Low		Low	Low	Low	

Table 3-2. Preclinical Studies

Study	Sample characteristics	Study design	Intervention	Donor	Measurement	Key findings and conclusions
Zhang et al. 2019 [28]	Chronic Stress Mice	Randomized Controlled Trial	FMT from wildtype (WT) and NLRP3 KO mice to chronic unpredictable stress (CUS) mice	WT and NLRP3 KO mice	SPT, FST, TST, and OFT	<ul style="list-style-type: none"> • Transplantation of the NLRP3 KO gut microbiota ameliorated CUS-induced depressive-like behaviors.
Li et al. 2019 [21]	Antibiotic treated 8 male WT and 8 male chronic unpredictable mild stress (CUMS) mice	Randomized Controlled Trial	Oral FMT for 2 weeks from WT and CUMS mice to antibiotic-treated WT and CUMS mice	8 WT mice and 8 CUMS mice	SPT, OFT, EPM, FST	<ul style="list-style-type: none"> • FMT of CUMS microbiota induced anxiety-like and depression-like behavior in the recipient mice
Lv et al. 2019 [22]	Antibiotic treated Male rats with and without CUS	Randomized Controlled Trial	3-day oral FMT from WT and CUMS mice to antibiotic-treated rats with and without CUS	WT and CUMS mice	SPT, OFT, EPM, FST	<ul style="list-style-type: none"> • Transplantation of CUMS Microbiome Induces Depression-Like Behaviors in Antibiotic-Treated Rats as shown via a decrease in time spent in the central area in the OFT and increased immobility in the TST
Xiao et al. 2018 [29]	6–8 week male C57BL/6 mice	Randomized Controlled Trial	14 days of saline or oral FMT from alcohol or water exposed mice to healthy control mice	Alcohol-exposed and water-exposed mice	FST and TST	<ul style="list-style-type: none"> • FMT from alcohol-exposed mice induced depressive behavior in the recipients, shown by significant results in FST and TST
						<ul style="list-style-type: none"> • Alcohol withdrawal induced symptoms were transmitted to healthy controls
				Two-month old		

Study	Sample characteristics	Study design	Intervention	Donor	Measurement	Key findings and conclusions
Yang et al. 2019 [68]	Antibiotic treated two-month-old male C57BL/6 mice	Randomized Controlled Trial	14 days of FMT from rats to antibiotic treated C57BL/6 mice	Sprague Dawley rats with and without anhedonia	Mechanical withdrawal test (MWT), Tail flick test (TFT), SPT, locomotion, TST, and FST	• Antibiotic administration significantly aggravated the MWT scores, latency of TFT, and depression-like behaviors.
						• FMT from rats with anhedonia significantly aggravated behavioral abnormalities, pain, depression-like, and anhedonia-like behaviors in recipient mice
						• Antibiotics-treated pseudogerm-free mice showed depression-like and anhedonia-like phenotype compared to control group, which were improved by FMT from mice without anhedonia.
Tillmann et al. 2019 [32]	24 adult male Flinders sensitive line (FSL) and 24 Flinders resistant line	Randomized Controlled Trial	FMT from FRL, saline, or FSL rats to FRL and FSL rats administered every third day over a 16-day period	FSL, FRL, or saline rats	OFT and FST	• Rats receiving FRL feces struggled less than saline-treated ones while there was no difference between FSL feces and saline or FSL and FRL feces.
						• Rats receiving FSL feces had significantly increased immobility compared with saline, whereas FRL feces did not differ from saline.

Study	Sample characteristics	Study design	Intervention	Donor	Measurement	Key findings and conclusions
						<ul style="list-style-type: none"> • No difference in immobility between FSL and FRL feces.
Langgartner et al. 2018 [25]	Male C57BL/6 N chronically stressed mice via chronic subordinate colony (CSC)	Randomized Controlled Trial	Repeated FMT from non-stressed single-housed control (SHC) mice	Non-stressed, SHC Male C57BL/6 N mice	OFT and open-field/novel object (OF/NO) test	<ul style="list-style-type: none"> • SHC feces transplantation had mild stress-protective effects as shown by an improvement of CSC-induced thymus atrophy, anxiety, systemic low-grade inflammation, and alterations in bone homeostasis. • CSC feces transplantation slightly aggravated CSC-induced systemic low-grade inflammation and alterations in bone homeostasis in SHC and/or CSC animals.
Jiang et al. 2020 [30]	18, 7-week old, antibiotic treated C57BL/6 J mice	Randomized Controlled Trial	FMT from 6 control, 6 alcohol-induced depression, and 6 alcohol-induced depression nicotinamide riboside (NR) treated mice after 3 weeks of antibiotic treatment	Control, alcohol-induced depression model, and alcohol-induced depression model NR-treated C57BL/6 J mice	SPT, FST, EPM, and Y-Maze	<ul style="list-style-type: none"> • Mice receiving FMT from alcohol induced depression model exhibited depression-like behaviour • Mice receiving FMT from control or NR mice did not exhibit depression-like behaviour.
Schmidt et al. 2020 [33]	Adult female Lewis rats	Randomized Double-Blind Sham Controlled Trial	FMT from healthy rats is given to rats with spinal cord injury (SCI)	Healthy, uninjured, adult female Lewis rats	Light-dark box, Cylinder test, SPT, EPM, OFT	<ul style="list-style-type: none"> • FMT from healthy rats significantly reduced depression and anxiety-like behaviour resulting from SCI in the elevated plus maze and light-dark box

Study	Sample characteristics	Study design	Intervention	Donor	Measurement	Key findings and conclusions
						(significantly more time in open arms of the maze and light box)
Siopi et al. 2020 [23]	8-week old, antibiotic-treated, GF C57BL/6 J mice	Randomized Controlled Trial	Antibiotic-treated GF mice receive FMT from 10 control, or 10 unpredictable chronic mild stress (USMS) mice	Control or UCMS mice	TST, FST, OFT, EPM, Light-dark Box	<ul style="list-style-type: none"> • FMT from UCMS mice resulted in despair-like behaviour in the TST and FST (increased immobility time in both)
Pearson-Leary et al. 2019 [24]	<p>Experimental Intruders: Singly housed male Sprague–Dawley rats</p>		FMT from vulnerable, resilient, and control rats delivered via oral gavage to naïve recipient rats once daily for 5 days.	SL/vulnerable, LL/resilient rats, or control rats	FST and SIT	<ul style="list-style-type: none"> • FMT from SL/vulnerable rats to naïve, non-stressed rats promotes some stress vulnerability
	<p>Residents: Male Long–Evans (LE) retired breeders (600–800 g) were used as residents.</p>					<ul style="list-style-type: none"> • No differences in time spent interacting in SIT between recipient groups suggesting no difference in anxiety-like behavior
						<ul style="list-style-type: none"> • Rats treated with vulnerable microbiota had increased passive depression-like behaviours (decreased latency to immobility, less time swimming, and increased time spent immobile) • SL/vulnerable microbiota treated rats also spent less time climbing, but this was not significant

Table 3-3. Preclinical Studies with Human Donors

Study	Sample characteristics	Study design	Intervention	Donor	Measurement	Key findings and conclusions
De Palma et al. 2019 [43]	141 GF NIH Swiss Mice	Randomized Controlled Trial	FMT from IBS patients and healthy donors to GF mice	4 Anxious IBS-D patients, 4 non-anxious IBS-D patients, Mean age: 40 years old; 5 healthy human controls (HHC), Mean age: 42 years	Donors: HAM-A	• FMT from anxious IBS-patients to mice produced anxiety behaviors in mice
					Recipients: Light-dark preference test and step-down test	• FMT from IBS patients with normal anxiety and from healthy controls to GF mice showed no significant anxious behaviors in GF mice
						• Akkermansia was associated with anxiety behaviors in mice
Hata et al. 2019 [42]	Germ-free (GF) BALB/c mice	Randomized Controlled Trial	Oral FMT with and without pre-treatment with live <i>Bacteroides vulgatus</i> to GF mice	4 AN patients, Mean age: 23 years, BMI 13.7; 4 HHC, Mean age: 25.3 years, BMI 21.6	Donors: DSM diagnosis of AN	• FMT from AN patients induces anxiety-like and compulsive behaviors in GF recipient mice and impairs body weight gain
					Recipients: Open Field and Marble Burying	• Pre-treatment with <i>B. vulgatus</i> attenuates compulsive behavior
Zhao et al. 2019 [39]	Male C57BL/6 J mice with antibiotic gut microbiota suppression, 6 weeks old	Randomized Controlled Trial	FMT via intragastric administration every other day for 13 days to antibiotic treated mice	Patients with and without alcoholism, Ages 35–40	Donors: ICD-10 diagnosis of alcoholism	• FMT from patients with alcoholism induced spontaneous alcohol dependence in mice
					Recipients: Open field test (OFT), alcohol preference test (APT), elevated plus maze test (EMT), tail suspension test	• FMT-Alc group exhibited anxiety-like and depression-like behaviors changes and significantly

Study	Sample characteristics	Study design	Intervention	Donor	Measurement	Key findings and conclusions
					(TST), and social interaction test (SIT),	declined social interaction behaviors
Huang et al. 2019 [36]	GF Mice	Randomized Controlled Trial	FMT from MDD patients and healthy controls to GF mice	5 MDD patients; 5 HHC	Donor: DSM-IV diagnosis of depression and HAM-D	• FMT from MDD patients resulted in significantly increased immobility times for the FST and TST
					Recipient: OFT, TST, forced swimming test (FST), buried food pellet test (BFP) and olfaction behavior test (via modified BFP)	• The center motion distance (OFT) also significantly decreased compared to controls
						• The latency for finding the object by depressed mice was significantly longer than that by healthy controls indicating impaired olfaction.
Kelly et al. 2016 [34]	Adult, male Sprague Dawley rats treated with antibiotic cocktail for 28 days	Randomized Controlled Trial	3-day pooled sample oral FMT from MDD patients and healthy controls to antibiotic treated rats, with booster inoculations given twice per week throughout the study.	Pooled fecal samples from 3 severely depressed MDD patients; pooled fecal samples from 3 HHC	Donor: Perceived stress scale (PSS), Beck Depression and Beck Anxiety Scales, HAM-D, etc.	• Rats receiving FMT from MDD patients demonstrated anhedonia-like behaviours as shown by a significant decrease in sucrose intake without affecting fluid intake in SPT
					Recipients: Sucrose preference test (SPT), OFT, EMT, FST	• Rats receiving FMT from MDD patients also exhibited anxiety-like behaviours as shown by a significant decrease in visits to the open arms in the EMT and a reduction in

Study	Sample characteristics	Study design	Intervention	Donor	Measurement	Key findings and conclusions
						<p>time spent in the centre in the OFT.</p> <ul style="list-style-type: none"> • In the forced swim test, there were no significant differences between the groups in immobility time, swimming, or climbing.
Xu et al. 2018 [40]	110 male C57BL/6 mice aged 4 to 5 weeks exposed to chronic ethanol	Randomized Controlled Trial	FMT 1: FMT at the end of chronic alcohol exposure period	3 HHC	Recipients: OFT, TST, FST, and APT	• FMT 1 could not alleviate alcohol-induced anxiety or depression.
			FMT 2: FMT at middle (6%) of alcohol exposure period.			• FMT 2 alleviated alcohol-induced depression in TST
			FMT 3: FMT at the beginning of whole exposure period			<ul style="list-style-type: none"> • FMT 3 modulated anxiety and significantly improved depression. • FMT 3 decreased Anxiety in OFT and significantly improved depression in TST. • No significant alcohol preference alternation in FMT-treated mice.
Zheng et al. 2016 [35]	Male GF Kunming mice and specific pathogen free (SPF) Kunming mice, 6–8 weeks old	Randomized Controlled Trial	Pooled sample FMT from MDD patients and HHC to GF mice	5 Male MDD patients, ages 26–61; 5 Male HHC, ages 29–6	Recipients: OFT, FST, TST	• Absence of gut microbiota in germ-free (GF) mice resulted in decreased immobility time in the FST compared to conventionally raised HC mice

Study	Sample characteristics	Study design	Intervention	Donor	Measurement	Key findings and conclusions
						<ul style="list-style-type: none"> • The gut microbiota compositions of MDD patients and HC were significantly different with MDD patients characterized by significant changes in the relative abundance of Firmicutes, Actinobacteria and Bacteroidetes. • FMT from MDD patients to GF mice resulted in depression-like behaviors compared to HC colonization • Weight was not significantly different between groups
Chen et al. 2020 [69]	ASD Model Mice	Randomized Controlled Trial	Pooled samples from Healthy Human donor gut bacteria (M + O) or cultured bacteria from original pooled healthy donor gut bacteria (M+ F)	Original healthy Human bacteria (M + O); in vitro cultured bacteria from healthy human donor (M + F)	OFT, Marble Burying Test, Self-grooming, Three-Chamber Social Test	<ul style="list-style-type: none"> • M + O spent significantly more time and had more entries in the OFT, significantly lower % of marbles burried, and significantly lower % of grooming time. • M + F had significantly lower % of marbles burried, and % of grooming time. • These results suggest that FMT from organic in vivo microbiota may be better at

Study	Sample characteristics	Study design	Intervention	Donor	Measurement	Key findings and conclusions
						alleviating depressive and anxiety-like behaviours, but that both in vivo and in vitro bacteria transplantation have beneficial properties.
Liu et al. 2020 [37]	18, 8-week old GF rats		Microbiota from healthy or depressed humans were transplanted into GF mice	Depressed or Healthy Humans between ages 18–60	Donors: 24+ points on HAM-D, DSM-5 diagnosis of MDD Recipients: FST, SPT	<ul style="list-style-type: none"> • Rats receiving depression microbiota exhibited depression-like behavior (immobility time in the forced swimming test was significantly higher than in control groups) • From the first week to the fourth week, the saccharine preference index was significantly lower in the depression microbiota group than that in the blank control and healthy microbiota groups

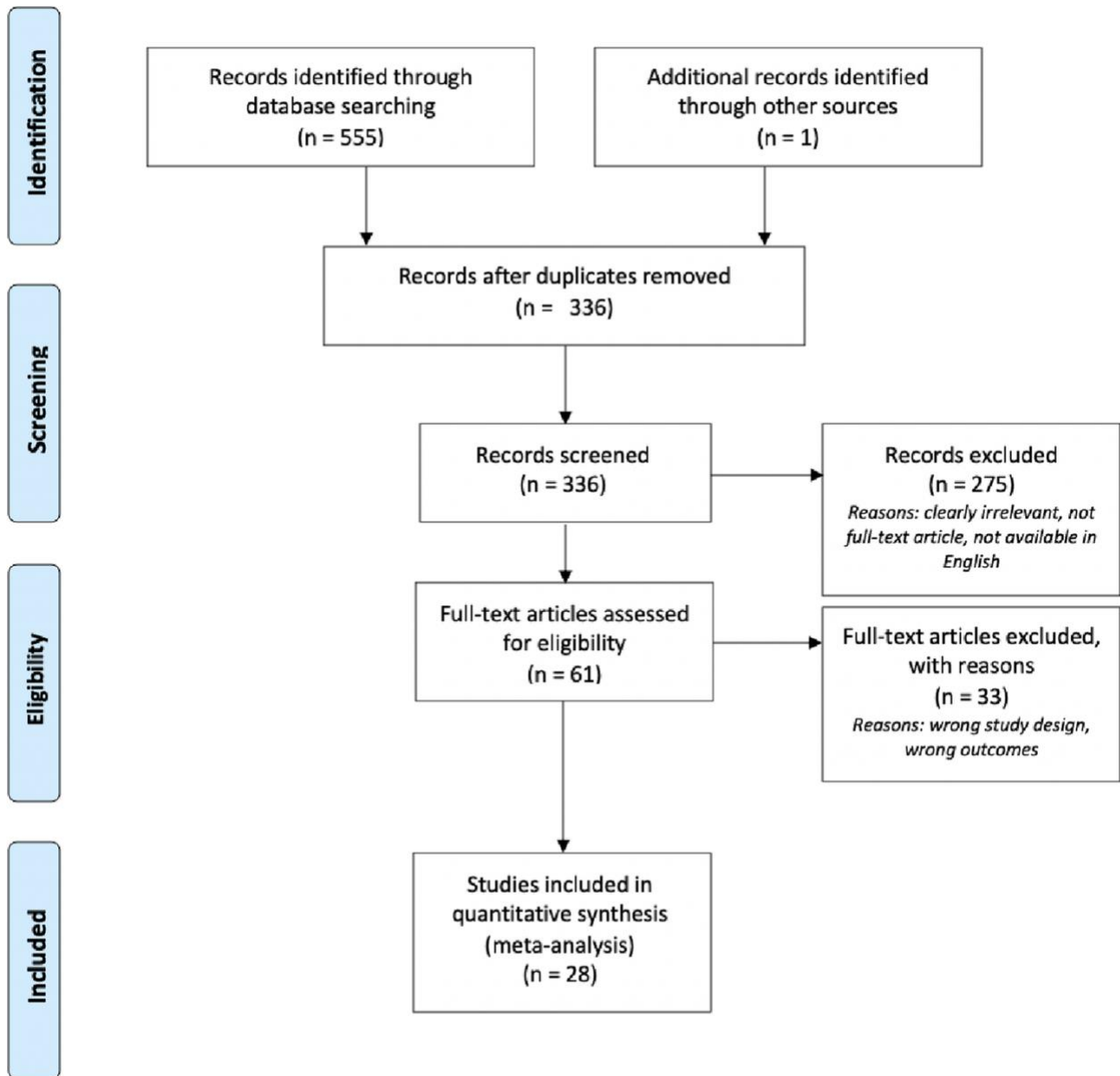
Table 3-4. Clinical studies

Study	Sample characteristics	Study design	Intervention	Donor	Measurement	Key findings and conclusions
Cai et al. 2019 [66]	1 female MDD patient, 79 years old	Pre- and post-intervention assessment	Single time FMT via gastroscop	6 year-old grandson	PHQ-9	• Six months after intervention PHQ-9 scores improved.
						• Significant increase in Firmicutes counts and Bacteroides significant reduced
De Clerq et al. 2019 [67]	1 female AN patient, 26 years old	Case report, pre- and post-intervention assessment	Single duodenal FMT	Unrelated female donor with BMI of 25	BMI, caloric intake	• Increase in BMI post-intervention
						• No significant differences in gut microbiota composition after FMT
Huang et al. 2019 [45]	30 (18 M, 12F) refractory IBS patients, Mean age: 44 years old	Pre- and post intervention assessment with 1, 3, and 6 month follow-ups	Two to three FMT procedures (done every other day) via colonoscopy	Healthy volunteers aged 8–35	IBS-QOL, IBS-SSS, GSRS, HAM-A and HAM-D	• Significantly improved GI symptoms and alleviated depression and anxiety as indicated by IBS-QOL, IBS-SSS, GSRS, HAM-A and HAM-D scores, 1 and 3 months post-FMT
						• Increase in Verrucomicrobia and Euryarchaeota at phyla level and increase in Methanobrevibacter and Akkermansia at the genus level, at 1 month after FMT compared to before FMT
Mazzawi et al. [46]	13 (9 M, 4F) IBS patients, Mean age: 32 years old	Open label, pilot study	Single duodenal FMT via gastroscop	Healthy donors, aged 20–42	IBS-SQ, IBS-SSS, EPQ-N-12, and HAD	• Scores of all questionnaires improved significantly at all follow-up time points and lasted up to 28 weeks
						• Patients' microbiota compositions became more similar to donors after FMT

Study	Sample characteristics	Study design	Intervention	Donor	Measurement	Key findings and conclusions
Mizuno et al. 2017 [44]	10 (7 M, 3F) refractory IBS patients, Mean age: 40.1 years old	Single arm, open label, non-randomized study with 12-week follow-up	Single time FMT via colonoscopy	Healthy relatives in second-degree relationship, Mean age: 52 years	HAM-A, HAM-D	• The HAM-D score significantly improved 4 weeks after FMT but returned to the baseline level at 12 weeks
						• When evaluated with HAM-A, the GI symptoms significantly improved from before FMT to 12 weeks after in responders, but not in non-responders
						• Significant increase in microbial diversity from before treatment to week 4
						• Significant relationship between diversity and response to treatment at week 4 but not before treatment
Xie et al. 2019 [47]	1 male MDD patient with alopecia and GI symptoms, 86 years old	Case report, pre- and post-intervention assessment	Six rounds of FMT via colonoscopy	22-year old healthy male donor	HAM-D	• Improved depressive symptoms
						• Improved appetite and no abdominal pain or distension, increased BMI.
						• Improved hair growth without any hair loss treatments
Kurokawa et al. 2018 [48]	17 (8 M, 9F) IBS patients, Mean age: 43.41	Single arm, non-randomized, open label, observational study	Single time FMT, via colonoscopy	Healthy relatives in second-degree relationship, Mean age: 51.41 years	HAM-D and subscale of sleep-related items, HAM-A, and QIDS	• Significant improvement in HAM-D total and sleep subscale score, HAM-A, and QIDS after FMT, at times even without GI symptoms improvement
						• Significant increase in microbiome diversity after FMT

Study	Sample characteristics	Study design	Intervention	Donor	Measurement	Key findings and conclusions
Johnsen et al. 2020 [49]	85 IBS (non-constipated) patients between 18 and 75 years of age	Double-blind, Randomized Controlled Trial, Parallel group	FMT (frozen or fresh) using health donors or using patient's own feces, delivered to cecum of IBS patients via colonoscope	Frozen or fresh feces from healthy donors	Fatigue Impact Scale (FIS), IBS-QoL, IBS-SSS	• Clinical effect on QoL and fatigue six months after treatment, with waning effect from six to twelve months,
						• Transient treatment effect seen in individuals with other functional disorders.
						• Absence of other self-reported functional disorders and presence of depression at baseline is suggested to predict a lasting effect of FMT in QoL and fatigue, respectively

Figure 3-1. Flow chart showing literature search and screening process using PRISMA process.



CHAPTER 4

NEUROBIOLOGICAL EFFECTS OF MICROBIAL TREATMENTS WITHIN PSYCHIATRY: A SYSTEMATIC REVIEW

4.1 Prologue

Through the reviews reported in previous chapters, it became clear that the majority of investigations into gut microbiome therapeutics in psychiatry focused on efficacy, safety, and tolerability. Though some physiological findings had been reported and discussed in the previous reviews, a review of the neurobiological findings from these investigations was needed for a better understanding of potential mechanisms of action and physiological effects of said therapeutics, and in preparation for the neuroimaging analysis to be reported in chapter 5.

4.2 Manuscript Information

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Relative contributions to manuscript: Cassandra Sgarbossa completed the initial search of the databases, adhering to the search strategy. William Redfearn and Dharmayu Desai independently assessed the titles and abstracts of records retrieved from a systematic search according to the identified inclusion and exclusion criteria. Maria Farid and Katherine Gallant completed the full-

text review. Any conflicts were resolved by a third-party, Cassandra Sgarbossa. Evan Forth completed the quality assessment characteristics and risk of bias. All authors contributed to the manuscript and approved of the submitted version.

4.3 Manuscript

4.3.1 Abstract

Objective: Though microbial interventions, such as probiotics and fecal microbiota transplantation, have had a growing body of evidence suggesting their efficacy in alleviating the symptoms of psychiatric illnesses, their exact mechanisms of action and impacts on the brain are still not fully characterised. The aim of this review is to compile and summaries the current literature regarding neural and neurobiological changes associated with microbial interventions in healthy and psychiatric populations. **Methods:** A systematic search of four databases was conducted using key terms related to neuroimaging, microbial interventions, and psychiatric illnesses. All results were then evaluated based on specific eligibility criteria. **Results:** 10 papers met the eligibility criteria, were included in this systematic review. Three of the five healthy control studies, and all five of the studies conducted within psychiatric populations observed significant neurobiological changes associated with probiotic intervention either in areas with psychiatric relevance, in the direction of a healthier profile, or correlated with improved psychiatric symptoms. **Conclusions:** The findings from the studies included in this review suggest that probiotic intervention may be associated with neurobiological changes, and that these changes could play a role in ameliorating psychiatric symptoms. More research is needed to replicate these findings, explore other psychiatric populations and microbial interventions, and

fully elucidate the mechanisms driving these neurobiological and clinical changes, but the findings from these studies are promising.

4.3.2 Introduction

While there has been a variety of treatment options that have existed throughout history as interventions for psychiatric disorders, common current treatments often include pharmacotherapy (e.g., antidepressant medications, mood stabilizers, antipsychotics, etc.) and/or psychotherapy (e.g., cognitive behavioural therapy) (1, 2). However, given the heterogenous nature of psychiatric disorders and unique variability between individuals, approximately 20 to 60% of patients can still experience treatment resistance (3). In efforts to optimize patient care and better understand the potential mechanisms involved with treatment response, there has been a push to explore novel and unconventional treatment options. Recently, microbial interventions targeting the gut microbiome (e.g., probiotics, fecal microbiota transplant [FMT], etc.) have gained traction and interest as potential target for treating psychiatric disorders (4, 5).

The gut microbiome is a complex ecosystem of living bacteria that exist within the human gut. In fact, the gut is home to approximately 100 trillion microbes, which is ten times larger in quantity than there are cells in the human body (6). While gut microbiome development is primarily critical during the first three years of life, it is continuously modified throughout life based on external influences such as geographical location, stress, medication use, and/or diet (7). Furthermore, despite Bacteroidetes and Firmicutes being the two predominant bacterial phyla found in healthy adult humans, external influences can still alter microbial composition over time and play a role in the development of dysbiosis (8). This becomes evident in clinical settings, as certain psychiatric disorders such as major depressive disorder (MDD), have been

associated with systematic inflammation (9, 10), gut dysbiosis (11,12), and altered gut microbiome composition (13, 14, 15).

The dynamic ability of the gut microbiome to influence psychological states and overall mood is suggested to be mediated by the Gut-Brain Axis (GBA), a bidirectional signaling pathway between the gastrointestinal (GI) tract and central nervous system (4). The GBA is essential in regulating various physiological and homeostatic processes within the body (16), while also aiding in the integration of communication from the nervous, immune, and GI systems (17). These processes by which the gut microbiome is able to modulate mood via the GBA, is still unclear. The nature of these processes is not mutually exclusive – but rather, they are an entanglement of systems interacting with one another. They interact with both direct and indirect pathways, such as endocrine (e.g. hypothalamic-pituitary-adrenal axis regulation) (18, 19), neural (e.g., vagus nerve interactions) (20, 21, 22), and immune pathways (e.g., involvement of inflammatory cytokines) (10, 23, 24).

Microbial interventions have long been used to treat various GI-related diseases, such as irritable bowel disease (IBD), clostridium difficile (CD) (25), and ulcerative colitis (UC) (26), with the purpose of repopulating and/or altering the gut microbiome through microbiota manipulation. These interventions often include but are not limited to: various bacteriotherapeutic options such as prebiotics, probiotics, and synbiotics (27, 28), FMT (26), and novel FMT alternatives (29). FMT, a well-documented treatment method that aims to restore microbial balance of the gut microbiome, involves the transfer of fecal matter from a healthy donor to the intestinal tract of an unwell recipient via colonoscopy. While traditionally used as treatment for CD infections (30) and IBD (31, 32), it has recently been investigated for its use in

treating psychiatric disorders (29, 33, 34), however more work is needed to confirm these findings and better understand FMT's role as a psychiatric therapeutic agent.

In addition to FMT, the use of probiotic supplementations for the management of psychiatric symptoms and mood has also been explored, due to their increased feasibility and cost-effective nature in comparison to other microbial interventions (35, 36, 37). Probiotics work by introducing live bacteria to the gut microbiome in efforts to maintain its health and functioning (17, 35). *Lactobacillus* and *Bifidobacterium* are amongst the most common genus found in probiotic supplementations targeted for use within psychiatry, due to their suggested influence on clinical symptoms and outcomes (38, 39, 40). As a treatment for depression, probiotics have been associated with improvements in cognitive performance, reduced neurodegenerative compounds (e.g., kynurenine) (41), improved depressive symptomology (39), and improved memory recall (42)

Neuroimaging use within psychiatry has been used to identify structural and functional characteristics associated with specific disorders and better understand any involved neural mechanisms (43). Structural techniques, such as computerized tomography (CT), voxel-based morphometry (VBM), magnetic resonance imaging (MRI), and diffusion tensor imaging (DTI) provides information on brain structure and size, visualization of grey and white matter composition, and gross brain abnormalities such as tumours (44). In contrast, functional techniques, such as functional magnetic resonance imaging (fMRI), positron emission tomography (PET), and electroencephalography (EEG), can measure the activity levels of different parts of the brain, which researchers and clinicians can associate with cognitive/behavioural tasks and various clinical predictors and outcome variables to guide research and treatment (44, 45, 46).

Recently, there has been a surge of neuroimaging use within psychiatry to help identify distinct pathophysiological mechanisms underlying various psychiatric disorders and treatments, as well as expound the etiologies of these disorders. The aim of this review is to further explore any associated neural and/or neurobiological changes of microbial interventions, within the scope of a psychiatric settings. This research serves to better understand the effect of microbial changes throughout the body, with massive potential and implications for optimizing patient health of individuals experiencing a psychiatric illness or mood disturbance.

4.3.3 Methods

4.3.3.1 Literature Search Strategy

This review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines for systematic reviews in unison with Covidence, a primary screening and data extraction tool (47). A total of four databases (MEDLINE, PsycINFO, EMBASE, and Web of Science) were utilized in the search strategy, including all relevant articles from inception to date of search on January 15th, 2025. Key search terms included: (magnetic resonance imaging OR MRI OR functional magnetic resonance imaging OR fMRI OR diffusion tensor imaging OR DTI OR positron emission tomography OR electroencephalography OR EEG OR computerized tomography OR CT OR functional near-infrared spectroscopy OR fNIRS OR ultrasound OR magnetoencephalography) AND (probiotic* OR prebiotic* OR postbiotic* OR psychobiotic* OR fecal microbiota transplant* OR FMT OR stool transplant OR bacteriotherapy OR microbe therapy OR microbe transfer) AND (depress* OR MDD OR bipolar* OR attention deficit hyperactivity disorder OR ADHD* OR autism* OR ASD OR

anxiety* OR mania OR schizo* OR obsessive compulsive disorder OR OCD OR posttraumatic stress disorder OR PTSD). The search yielded 745 studies after 104 duplicates were removed. After abstract screening, 25 studies were assessed for eligibility through full-text review and 15 were excluded for either: no assessment of psychiatric symptoms, abstract only, no use of imaging, or wrong intervention. The remaining 10 studies passed all criteria and were included in the review. For a detailed overview of the screening process, please see Figure 4-1.

4.3.3.2 Eligibility Criteria

The following inclusion criteria were required to be eligible for the review: (1) Must utilize a neuroimaging technique either a) during intervention; b) after intervention; or c) during and after intervention; (2) Must assess for symptoms of psychiatric disorders; (3) Subjects must have undergone a form of microbial-based intervention; (4) Studies written in English and published in a peer-reviewed journal. No other criteria were used to exclude studies from review.

4.3.3.3 Study Selection

Author C.S. completed all literature searches. Authors W.R. and D.D completed the abstract screening, M.F. and K.G. completed full-text review, and any conflicts between reviewers was resolved by a third party, C.S. Author A.G. conducted data extraction and E.F. completed study quality assessment.

4.3.3.4 Study Quality

Quality assessment of articles was completed using Covidence's built-in, Cochrane Handbook for Systematic Reviews of Interventions, Risk of Bias (RoB) template. The Cochrane RoB tool assesses the risk of bias for the following domains: sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, and

incomplete outcome data. Most studies presented with a low level of bias. Studies where there was no mention of blinding to participants, personnel, outcome assessors, or allocation of treatment, were assigned a “high” RoB judgment. A detailed summary of the quality assessment can be found in Table 4-1.

4.3.4 Results

4.3.4.1 Study Characteristics

Of the 10 papers that passed all inclusion criteria and were included for data extraction and analysis, 5 included studies that involved healthy participants and the remaining 5 involved participants with psychiatric disorders. All studies used probiotics containing bacteria from the *Bifidobacterium* and *Lactobacillus* genera along with various other strains and species as the intervention. The neuroimaging techniques used in these studies included MRI (n=2) (48, 49), fMRI (n=5) (39, 42, 50, 51, 52), voxel-based morphometry (n=2) (39, 48), and EEG (n=3) (53, 54, 55). Healthy control studies included a total of 190 participants, with sample sizes ranging from n=22 to n=59 and an average sample size of 38. The psychiatric-focused studies involved 134 participants in total, including individuals with autism spectrum disorder (ASD) (n=63), MDD (n=44), and irritable bowel syndrome (IBS) with mild to moderate depression and/or anxiety symptoms (n=44). While there are three reported studies for MDD, it is important to note that all of them are reporting on the same patient population. The initial work was published by Schaub and colleagues (2022), with separate secondary analyses conducted by Schneider and colleagues (2023) and Yamanbaeva and colleagues (2023). Given that each of these studies reported unique findings, paired with the limited available neuroimaging data associated with

probiotic supplementation within psychiatry, the decision was made to retain these studies in the review. A detailed summary of study characteristics can be found in Table 4-2.

4.3.4.2 Healthy Indication

In the work completed by Allen and colleagues (2016), healthy male volunteers (n=22) received placebo for four weeks before receiving probiotic intervention for another four weeks. Through the use of EEG data, they found probiotic intervention to be associated with an increase in midline frontal (Fz) mobility and a decrease in midline central (Cz) theta power. These EEG changes were correlated with subtle improvements in visuospatial memory as assessed by the Cambridge Neuropsychological Test Automated Battery (53).

Papalini and colleagues (2019) had healthy participants (n=58) receive either probiotic intervention (n=29) or placebo (n=29) for four weeks. They reported that the probiotic intervention group displayed an increased buffer to the effects of stress on working memory performance, in relation to the placebo group. Physical stress was induced using the Social Evaluated Cold Pressor Test, where participants submerged their hand in cold water for up to 3 minutes. Psychological stress was induced by having an unknown researcher display neutral and socially distant behaviour, and instructing participants to look into a camera during the cold-water test to have their facial expressions recorded. Participants underwent the digit span test to evaluate their working memory before and after the Social Evaluated Cold Pressor Test, pre- and post-intervention. Participants in the probiotic group were less negatively affected by stress, than the placebo group. This increased buffer effect was especially seen in individuals with probiotic-induced decreases in activity in the right prefrontal cortex during stress-related working memory tests (50).

Lastly, in the study conducted by Rode and colleagues (2022), healthy participants (n=22) were given either a probiotic mixture or placebo for 4 weeks, followed by a 4-week washout period, and then switching to the other arm (either probiotic or placebo depending on which they started with), for an additional 4 weeks. They reported probiotic intervention to be associated with a significant increase in the functional connectivity between the default mode network and postcentral gyrus and superior parietal lobule, as well as between the language network and the middle temporal gyrus, inferior temporal gyrus, and lateral occipital cortex. Significantly reduced functional connectivity was observed after probiotic intervention between the left supramarginal gyrus (within the salience network) and the postcentral gyrus, as well as between the right supramarginal gyrus (within the salience network) and the brain stem, precuneus cortex, cerebellum, and supracalcarine cortex. Significantly reduced functional connectivity was also observed between the frontoparietal network and the middle frontal and precentral gyri (48).

In the two remaining studies, Kelly and colleagues (2017) as well as Ascone and colleagues (2022) both reported no significant differences between the probiotic and placebo groups after probiotic intervention (49, 54).

4.3.4.3 Psychiatric Indication

As previously mentioned, three of the MDD studies had reported results on the same population of participants, due to Schneider and colleagues (2023) and Yamanbaeva and colleagues (2023) reporting secondary analyses of the work by Schaub and colleagues (2022) (39, 42, 51). In the original study by Schaub and colleagues (2022), participants received either probiotic or placebo (in addition to treatment as usual) for a period of 31 days, with a follow-up assessment 4 weeks after the intervention. They found a significant increase in grey matter volume in the calcarine sulcus extending into the lingual gyrus after probiotic intervention, when

compared to the placebo group. When looking at activity changes pre- and post-intervention, there was a significant decrease in right and left putamen activation during neutral face processing, in the probiotic group. There were no significant activation changes in this region, for the placebo group. For clinical results, both the probiotic group and the placebo group had a significant decrease in HAM-D scores over time, however the effect was stronger in the probiotic group (39).

A secondary analysis conducted by Schneider and colleagues (2023) found a decrease in left hippocampus activation in the probiotic group after intervention, in a working memory task. When the activity changes in the left hippocampus were correlated with reaction time during the task, it was found that decreased hippocampus activity in the probiotic group was correlated with a decreased reaction time during the task. In contrast, increased hippocampus activity in the placebo group was correlated with a decreased reaction time during the task (42).

Another secondary analysis conducted by Yamanbaeva and colleagues (2023) found the probiotic group to have significantly higher fractional anisotropy (FA) in the uncinate fasciculus (UF), as compared to placebo. The probiotic group also maintained a stable mean diffusivity during and after treatment, whereas the placebo group had a significant increase in mean diffusivity post-treatment. When examining resting state functional connectivity (rsFC) in the probiotic group, increased connectivity was observed between the subcallosal cortex, left temporal pole, right and left hippocampus, and right and left amygdala, to a cluster in the precuneus. Increased connectivity was also observed between the left orbitofrontal cortex and a cluster in the left superior parietal lobule extending to the left posterior supramarginal gyrus. Decreased connectivity was observed between the subcallosal cortex, left hippocampus, and right amygdala, with this cluster. When looking at blood perfusion, they reported the mean

cerebral blood flow (CBF) in the hippocampus to be significantly higher in the placebo group than the probiotic group (51).

A study by Pinot-Sanchez and colleagues (2017) examined the use of a probiotic product in a population with IBS and diarrhea or a mixed-stool pattern, and mild to moderate anxiety and/or depression (n=44). Participants were randomized to receive either probiotic or placebo for a period of six weeks, with fMRI data collected both before and after treatment. Post-intervention, the probiotic group displayed reduced activation of the amygdala and frontal and temporal cortices, and increased activation of occipital regions in response to fear stimuli, when compared to the placebo group. Within the probiotic group, reduced amygdala activation was significantly correlated with decreased depression scores and was more likely to occur in patients with adequate relief of IBS symptoms, than those without it (52).

Lastly, Billeci and colleagues (2023) investigated the use of a probiotic mixture in children aged 18 – 72 months with ASD (n=63). They were given either probiotic or placebo for 6 months, with EEG measures conducted pre- and post-treatment for changes in power, coherence, and asymmetry. In the probiotic group, both beta and gamma bands displayed a decrease in power in the right and left frontopolar regions and an increase in that frontopolar coherence. The probiotic group also displayed a decrease in frontal asymmetry in delta band, while the placebo group displayed an increase in frontopolar asymmetry in alpha band. Lower raw field potential power in gamma band was found to be correlated with a lower number of caregiver-reported repetitive behaviors (e.g., stereotyped and/or self-injurious behaviour, etc.), while higher frontopolar coherence in beta and gamma bands was correlated with higher writing skill scores on a semi-structured interview with caregivers. In addition to this, children with

lower levels of tumor necrosis factor alpha (TNF-a) post-intervention also showed higher frontopolar coherence in gamma band.

4.3.5 Discussion

4.3.5.1 Main Findings

Ten studies investigating the effects of probiotic intervention on neurobiological structures and functions were included in this systematic review. All studies used probiotic formulations containing primarily bacteria from the *Lactobacillus* and *Bifidobacterium* genera. Of the five studies conducted with healthy populations, three found significant neurobiological changes after probiotic intervention (48, 50, 53), specifically within domains such as the default mode network, working memory performance areas, and brain activity measured by EEG's. While these changes maintain relevance within the field of psychiatry, there were no significant differences in psychiatric symptoms between the probiotic and placebo groups. The remaining two healthy indication studies found no significant differences between the probiotic and placebo groups (49, 54). As for the five studies conducted with psychiatric populations, all found significant neurobiological changes resulting from probiotic interventions that were either in the direction of a healthier profile or correlated with improved psychiatric symptoms (39, 42, 51, 52, 55).

While two studies with healthy populations did not report any significant changes between probiotic and placebo groups (49, 54), other work reported quite the opposite. Rode (2022) found probiotic intervention in healthy individuals to be associated with decreased grey matter volume in the left supramarginal gyrus, decreased functional connectivity between both supramarginal gyri, and multiple other brain regions associated with emotional regulation, the

salience network, and the default mode network (48). They postulate that the decrease in grey matter and functional connectivity could indicate higher brain efficiency. Interestingly, other work has found that increased grey matter in the inferior parietal lobule, which contains the supramarginal gyrus, has been observed in first episode treatment-naïve individuals with schizophrenia, when compared to healthy controls (56). Alterations in the default mode and salience networks have also been observed in many other mood and anxiety-related disorders (57, 58), suggesting the findings by Rode (2022) to have clear relevance in the context of psychiatric illnesses and disorders (48).

If the focus shifts to cognition, the studies conducted by Allen and colleagues (2016) as well as Papalini and colleagues (2019) both offer intriguing insights. Allen and colleagues (2016) observed an increase in EEG FZ mobility, a marker of prefrontal cortex activity, and a reduction in Cz theta power, correlating with improved memory performance after probiotic intervention (53). Similarly, Papalini and colleagues (2019) found that probiotics provided an increased resilience against stress-related challenges in working memory, specifically for those showing decreased activity in the right prefrontal cortex during cognitive control tasks (50). Both findings are relevant within a psychiatric context, as decreased prefrontal cortex activity and compromised working memory have both been observed in other disorders such as PTSD, bipolar disorder (59), manic symptoms (60), and schizophrenia (61, 62). Given that the prefrontal cortex (PFC) is crucial for its involvement in executive functioning and emotional regulation, it is without surprise that PFC activity is frequently hypoactive both at rest (62) and under stress (63), in psychiatric populations. Utilizing microbial treatment strategies such as probiotics, may aid in optimizing PFC functioning and serve as a tool to address any cognitive and/or emotional deficits associated with these disorders.

In psychiatric populations, probiotics continue to appear to have promising effects. Previous work has documented that individuals with depression can exhibit hyperactivity in structures associated with the limbic system, such as the putamen region and amygdala (64). Given these structures' involvement with facial expression recognition (65), it is postulated that hyperactivity in this area could contribute to a tendency to interpret neutral faces, as more negative and/or threatening (66, 67). Interestingly, Schaub and colleagues (2022) reported a decrease in putamen activation following probiotic intervention, while viewing neutral faces in a population with depression. The authors suggested these findings could be indicative of individuals experiencing a more balanced emotional response, interpreting neutral faces less negatively, or reacting less intensely to negative stimuli. Though these conclusions cannot be certain, the overall decrease in putamen activation serves as a marker for identifying neurobiological changes associated with both the disorder and response to probiotics. Similarly, Schnieder and colleagues (2023) reported reduced hippocampus activation during working memory tasks in the same population, after probiotic intervention, whereas the placebo group had an increase in hippocampal activation (42). This is aligned with other work done in the field, as the hippocampal region has been noted to be hyperactive during rest in a population with depression (68). While most of hippocampal work has been done through a structural lens, such as identifying volumetric changes and correlating it to clinical outcomes (69), structural data collection and analysis is beginning to catch up. Smith and colleagues (2017) also reported similar findings with a working memory task, where vortioxetine produced a reduction in hippocampal activity for both individuals with MDD and health controls, as compared to placebo (70). Yamanbaeva and colleagues (2023) provided further insights into the effects of probiotics on brain function, by observing a stabilizing effect of mean diffusivity in the uncinate fasciculus

for participants who received probiotics, whereas the placebo group experienced an increase. They also reported changes in rsFC between limbic structures and other brain regions, with an increase in connections to the precuneus and decrease in connections to the left superior parietal lobule (51).

Pinto-Sanchez and colleagues (2017) examined the use of probiotics in a population with IBS. They noted reduced neural responses to negative stimuli in the amygdala and fronto-limbic system, which both play essential roles in the brains' emotional regulation, memory, and decision making (52). Furthermore, Billeci and colleagues (2023) documented EEG changes towards a neuro-typical profile in preschoolers with ASD, post-probiotic intervention. These changes included decreased power in frontopolar regions and increased coherence in beta and gamma bands, which may reflect an improvement and restoration in the imbalance between excitatory and inhibitory neurons, as well as a change in brain connectivity towards a typical pattern (55).

4.3.5.2 Limitations

Some key limitations should be considered when interpreting the findings of this review. Firstly, there is a clear lack of literature regarding neurobiological changes associated with probiotic administration and psychiatric disorders, given that only five studies maintained a psychiatric scope. While this was anticipated due to the novelty of using microbial treatments for use within psychiatry, it is impossible to draw any firm conclusions on the influence of probiotics on specific disorders, such as depression. Additionally, even with these studies, they had relatively small sample sizes ranging from 32-47 individuals. This can be common for study designs involving frequent and/or numerous study assessments (e.g., neuroimaging, clinical scales, etc.), due to the increased burden on participants and associated inconsistency compliance. Furthermore, most studies maintained a relatively short intervention period and

lacked in-depth, long-term, evaluations after probiotic treatment. A common follow-up timepoint is often set at 4-weeks post-treatment, however that is hardly sufficient to observe any long-term significant changes in brain structure and function. While some findings demonstrated a positive effect on psychological symptoms and cognition following probiotic treatment, the extent of that sustained improvement, requires further investigation.

In the Schaub and colleagues (2022) study, they combined probiotic supplementation with standard treatment as usual for depression. While using probiotics as an add-on treatment is another necessary step towards understanding how microbial treatments influence mood, it also poses as a limitation when attempting to uncover any associated neural effects of probiotics. It raises the question as to whether any observed changes are in response to solely probiotic administration, antidepressant administration, or a combination of both treatments. This interplay between concurrent probiotic and antidepressant use needs further consideration, as some antidepressants have been found to have their own independent effects on the gut microbiome (71). Lastly, each study included in this review used different probiotic interventions. Though most probiotics used in these studies contained bacteria from the *Lactobacillus* and *Bifidobacterium* genera, the specific species, strains, doses, and mix of bacteria differed between each probiotic intervention. This makes it difficult to directly compare the effects of probiotics and inherently limits the generalizability of the findings.

4.3.6 Future Directions

This systematic review provides an overview of the current structural and functional changes correlated with probiotic treatment in healthy and psychiatric populations shown by various neuroimaging techniques. Most studies demonstrated that probiotic intervention was correlated with changes in brain structures, networks, or functioning that have been associated

with various psychiatric disorders (57, 58, 68). However, these results are still far and few. Further integration of neuroimaging use within double-blind randomized controlled trials with large and diverse sample sizes, along with in-depth follow-up periods, is necessary to establish the relationships between probiotic treatment, psychiatric symptoms, and neurobiological changes and/or biomarkers.

In terms of precision medicine, establishing biomarkers and incorporating their use into clinical psychiatric practice can aid in confirming diagnoses, predicting treatment outcomes, and better understanding the prognosis of disorders. Utilizing personalized approaches to patient care excels past the current status of their psychiatric state, as it integrates the unique variability of each individuals' genetic makeup, environmental factors, and lifestyle differences (72). Such information is invaluable for the continued development and improvement of effective treatment guidelines and medications within psychiatry. Given the novelty of using microbial therapeutics for mood-related changes and disturbances, neuroimaging serves as a potential tool to understand how gut-focused treatments can influence brain function and behaviour.

4.3.7 References

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Table 4-1. Risk of Bias (RoB) Assessment

Study	Sequence generation		Allocation concealment		Blinding of participants and personnel		Blinding of outcome assessment		Incomplete outcome data	
	RoB	Comments	RoB	Comments	RoB	Comments	RoB	Comments	RoB	Comments
Rode et al., 2022	Low	Participants were randomly assigned to treatment groups using a computerized randomization list and block randomization.	Low	Study was double-blind, all group assignment and sachet labeling was done by a university staff member not involved with the study. The study was blinded for all participants and staff.	Low	Study was double-blind, all group assignment and sachet labeling was done by a university staff member not involved with the study. The study was blinded for all participants and staff.	Low	Study was double-blind, all group assignment and sachet labeling was done by a university staff member not involved with the study. The study was blinded for all participants and staff.	Low	Subjects that were excluded were documented with reasoning.
Ascone et al., 2022	Low	Randomization was via a list created with a random sequence generator.	Low	Assignment was done by a third person who was unrelated to the study.	Low	Assessors and participants were blind to group allocation. The randomisation list was given to the first author after data collection was finished.	Low	Assessors and participants were blind to group allocation. The randomisation list was given to the first author after data collection was finished.	Low	Subjects that were excluded were documented with reasoning.
Allen et al., 2016	High	All participants received placebo first then probiotic intervention. No mention of randomization of allocation sequencing.	High	No mention of allocation concealment	High	No mention of blinding	High	No mention of blinding	Low	Subjects that were excluded were documented with reasoning.
Kelly et al., 2017	Low	Used a randomized placebo-controlled cross-over	High	No mention of allocation concealment	High	No mention of blinding	High	No mention of blinding	Low	Subjects who were excluded were

		repeated measures design but no mention as to how allocation was randomized.								documented with reasoning.
Papalini et al., 2019	Low	Participants were randomized using a computer-generated block randomization scheme by Winclone.	Low	No research personnel involved with participants could adjust the randomization or discern what products participants were receiving.	Low	No research personnel involved with participants could adjust the randomization or discern what products participants were receiving.	Low	No research personnel involved with participants could adjust the randomization or discern what products participants were receiving.	Low	Subjects who were excluded were documented with reasoning.
Billeci et al., 2023	Low	Random independent block allocation using a randomized sequence with a random order of interventions.	Low	Allocation information was sealed in envelopes	Low	Subjects, caregivers, all research investigators and all outcome assessors were blinded to treatment group assignment of all subjects till the end of data collection and analysis.	Low	Subjects, caregivers, all research investigators and all outcome assessors were blinded to treatment group assignment of all subjects till the end of data collection and analysis.	Low	Subjects who were excluded were documented with reasoning.
Schaub et al., 2022	Low	Block randomization was performed in a 1:1 ratio by an independent researcher using a computer-based randomization algorithm to avoid systematic biases	Low	States that investigators and assessors were blinded during data collection and analysis, but the details for how this was done are not stated	Low	States that investigators and assessors were blinded during data collection and analysis, but the details for how this was done are not stated		States that investigators and assessors were blinded during data collection and analysis, but the details for how this was done are not stated	Low	Subjects that were excluded were documented with reasoning.

Schneider et al., 2023	Low	Block randomization was performed in a 1:1 ratio by an independent researcher using a computer-based randomization algorithm to avoid systematic biases	Low	States that investigators and assessors were blinded during data collection and analysis, but the details for how this was done are not stated	Low	States that investigators and assessors were blinded during data collection and analysis, but the details for how this was done are not stated	Low	States that investigators and assessors were blinded during data collection and analysis, but the details for how this was done are not stated	Low	Subjects that were excluded were documented with reasoning.
Yamanbaeva et al., 2023	Low	Block randomization was performed in a 1:1 ratio by an independent researcher using a computer-based randomization algorithm to avoid systematic biases	Low	States that investigators and assessors were blinded during data collection and analysis, but the details for how this was done are not stated	Low	States that investigators and assessors were blinded during data collection and analysis, but the details for how this was done are not stated	Low	States that investigators and assessors were blinded during data collection and analysis, but the details for how this was done are not stated	Low	Subjects that were excluded were documented with reasoning.
Pinto-Sanchez et al., 2017	Low	Block randomization stratified by gender and IBS status using a computer program	Low	Allocation codes were sealed in opaque envelope. Treatment allocation was concealed from participants and study staff	Low	Treatment allocation was concealed from participants and study staff. Treatment products were indistinguishable in terms of package, color, taste, and consistency, and were blinded to subjects, investigators and support staff.	Low	Treatment allocation was concealed from participants and study staff. Treatment products were indistinguishable in terms of package, color, taste, and consistency, and were blinded to subjects, investigators and support staff.	Low	Subjects that were excluded were documented with reasoning.

Table 4-2. Study Characteristics

Study	Sample			Primary indication	Study design	Intervention	Timeline	Imaging measures	Clinical measures	Results
	Sample size	Age (Mean)	Sex							
Healthy indication										
Rode et al., 2022	n=22	24.2	16F, 6M	Healthy controls	RCT	Probiotic (containing: Bifidobacterium longum, Lactobacillus helveticus, and Lactiplantibacillus plantarum) vs. Placebo	4 weeks of treatment, 4 weeks of washout prior to crossover, then 4 weeks of alternate treatment.	MRI, VBM	EQ-5D-5L, HADS, PSS, STAI, KSD, DOW	Probiotic intervention resulted in significant functional connectivity changes in the default mode, salience, and frontoparietal networks, as compared to placebo. Psychological symptoms improved non-significantly, after probiotic intervention.
Ascone et al., 2022	n=59	27.1	33F, 26M	Healthy controls	RCT	Probiotic (containing: Lactobacillus paracasei, Lactobacillus plantarum, Lactobacillus acidophilus, Lactobacillus helveticus, Bifidobacterium lactis, Bifidobacterium breve, and Streptococcus thermophilus) vs. Placebo	4 weeks of treatment	MRI	BDI-II-R, BSI, PSS, RSQ	No significant changes in hippocampal grey matter and functional connectivity. No significant group x time interactions for whole-brain grey or white matter. No significant changes in psychiatric symptoms.
Allen et al., 2016	n=22	25.5	All males	Healthy males	RCT	Probiotic (containing: Bifidobacterium longum) vs. Placebo	4 weeks of placebo, followed by 4 weeks of probiotics treatment.	EEG	CPSS	Daily stress levels were lower during probiotic intervention but returned to elevated levels during the 2-week follow-up. For EEG, Fz mobility differed significantly across conditions and was significantly higher post-probiotic treatment. Cz theta power was significantly lower post-probiotic treatment,

										as compared with post-placebo.
Kelly et al., 2017	n=29	24.59	All males	Healthy males	RCT	Probiotic (containing: Lactobacillus rhamnosus) vs. Placebo	8 weeks of treatment	EEG	BDI, PSQI, PSS, SAI, TAI, SCL-90	No significant differences between probiotic and placebo group.
Papalini et al., 2019	n=58	21.5	All females	Healthy females	RCT	Probiotic (containing: Bifidobacterium bifidum, Bifidobacterium lactis, Lactobacillus acidophilus, Lactobacillus brevis, Lactobacillus casei, Lactobacillus salivarius, and Lactococcus lactis) vs. Placebo	4 weeks of treatment	fMRI	BDI, LEIDS-R, Emotional face-word Stroop paradigm, Color-word Stroop paradigm	With stress induction, the probiotic group demonstrated a significant increase in working memory performance after supplementation, as compared to placebo.
Psychiatric indication										
Billeci et al., 2023	n=46	46.5 months	11F, 35M	ASD	RCT	Probiotic (containing: Streptococcus thermophilus, Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium infantis, Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus paracasei, and Lactobacillus delbrueckii subsp. Bulgaricus) vs. Placebo	6 months of treatment	EEG	GSI, ADOS-2 CARS, SCQ, RBS-R, CBCL, GMDS-R, VABS-II, CDI	In the probiotic group, there was a decrease of power in frontopolar regions in beta and gamma bands and increased coherence in beta and gamma bands with a shift in frontal asymmetry. EEG measures were significantly correlated with clinical measures, as there was a significant association between frontopolar coherence in the beta and gamma bands and the "writing skills" domain of the VABS-II.
Schaub et al., 2022	n=47	39.1	27F, 20M	MDD	RCT	Probiotic (containing: Streptococcus thermophilus, Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium infantis, Lactobacillus acidophilus, Lactobacillus plantarum,	31 days of treatment	fMRI, VBM	HAM-D	There was a significant decrease in HAM-D scores over time, with a stronger effect in the probiotic group in comparison to the placebo group. No significant effects in grey matter volume, however the probiotics

						Lactobacillus paracasei, Lactobacillus delbrueckii subsp. Bulgaricus) vs. Placebo				group demonstrated increased grey matter volume in the calcarine sulcus after intervention, in comparison to placebo. Putamen activation in response to neutral faces had a significant decrease after probiotic intervention, in comparison to placebo.
Schneider et al., 2023	n=47	39.1	27F, 20M	MDD	RCT	Probiotic (containing: Streptococcus thermophilus, Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium infantis, Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus paracasei, Lactobacillus delbrueckii subsp. Bulgaricus) vs. Placebo	31 days of treatment	fMRI	VLMT, Corsi Block Tapping Test, Trail Making Test	Immediate recall in the VLMT significantly improved after probiotic intervention. There was also a significant difference in hippocampus activation during working memory processing in the probiotic group compared to placebo. Other measures did not reveal significant changes.
Yamanbaeva et al., 2023	n=47	39.1	27F, 20M	MDD	RCT	Probiotic (containing Streptococcus thermophilus, Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium infantis, Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus para-casei, Lactobacillus delbrueckii subsp. Bulgaricus) vs. Placebo	31 days of treatment	fMRI	HAM-D	Probiotics maintained mean diffusivity in the left uncinate fasciculus and altered resting-state functional connectivity (rsFC) between limbic structures and the precuneus. A cluster in the left superior parietal lobule showed altered rsFC to the subcallosal cortex, left orbitofrontal cortex, and limbic structures after probiotic intervention. Decreases in rsFC from the right amygdala to the cluster in the superior parietal lobule were related to decreased in depressive symptoms.

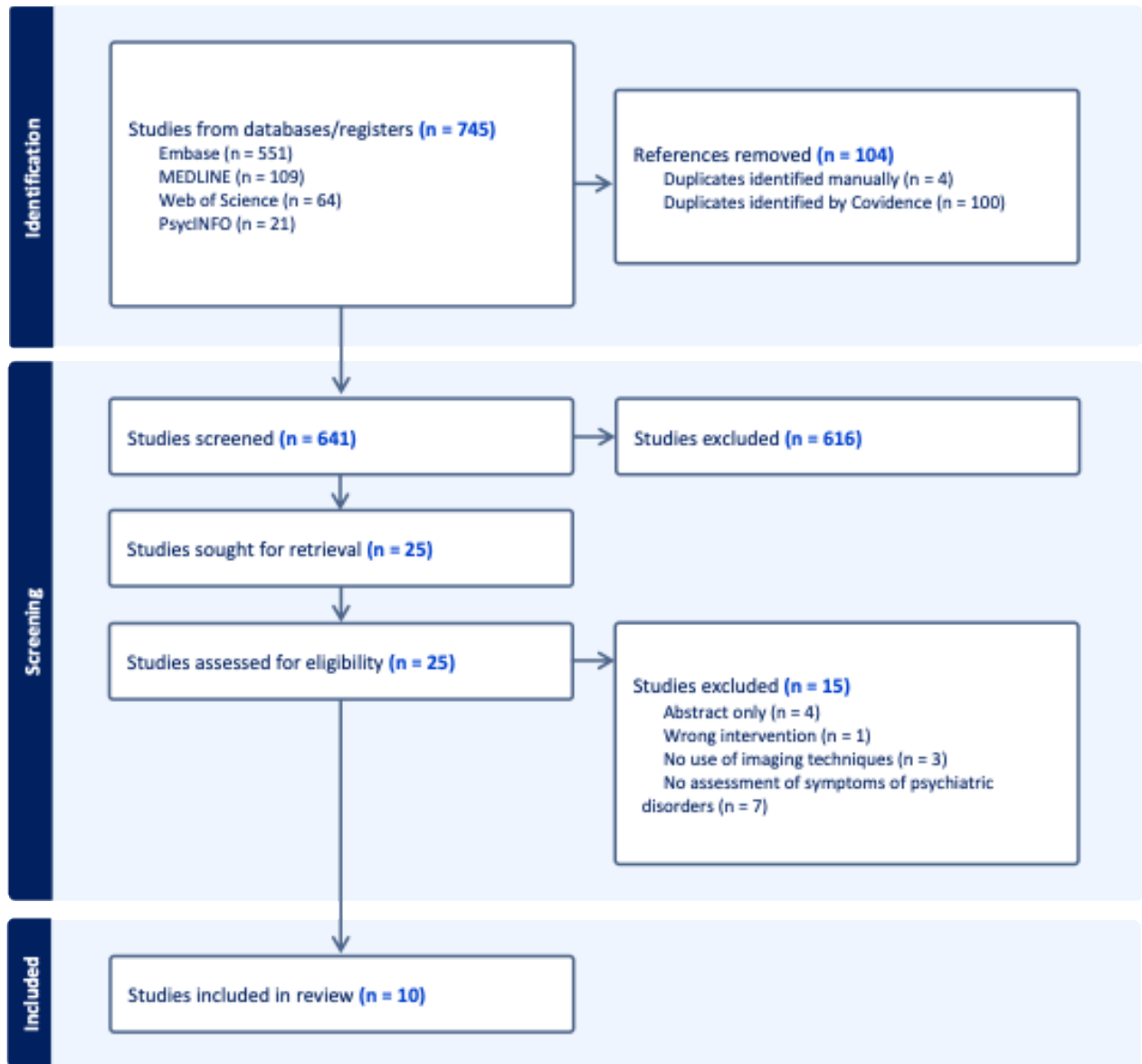
Pinto-Sanchez et al., 2017	n=44	43.25 (median)	24F, 20M	IBS	RCT	Probiotic (containing: Bifidobacterium longum) vs. Placebo	6 weeks of treatment	fMRI	HAD, STAI, Birmingham IBS Score, Bristol stool scale, self-reported IBS improvement, SF-36, PHQ-15	At week 6, 64% of probiotic participants and 32% of placebo participants had a reduction in HAD scores. Probiotics had no significant effects on anxiety or IBS symptoms. Participants in the probiotic group had a mean increase in QoL scores (SF-36) as compared to the placebo group. The probiotic group also displayed reduced activity in response to negative emotional stimuli in the amygdala and fronto-limbic regions, as compared to placebo.
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^aADOS-2: Autism Diagnostic Observation Schedule – Second Version; ASD: autism spectrum disorder; BDI: Beck Depression

Inventory; BDI-II-R: Beck Depression Inventory (2e) – Revised; BSI: Brief Symptom Inventory; CARS: Childhood Autism Rating Scale; CBCL: Child Behavior Checklist; CDI: McArthur-Bates Communicative Development Inventories; CPSS: Cohen Perceived Stress Scale; Cz: midline central; DOW: Diary of Workload; EEG: electroencephalography; EQ-5D-5L: -; fMRI: functional magnetic resonance imaging; Fz: midline frontal; GMDS-R: Griffiths Mental Development Scales - Extended Revised; GSI: Gastrointestinal Severity Index; HAD: Hospital Anxiety and Depression scale; HAM-D: Hamilton Depression Rating Scale; IBS: irritable bowel syndrome; KSD: Karolinska Sleep Diary; LEIDS-R: Leiden Index of Depression Sensitivity - Revised; MRI: magnetic resonance imaging; PHQ-15: Patient Health Questionnaire-15; PSS: Perceived Stress Scale; QoL: quality of life; RBS-R: Repetitive Behavior Scale-Revised; RCT: randomized controlled trial; RSQ: Response Styles Questionnaire; SAI: State Anxiety Inventory; SCL-90: Symptom Checklist-90; SCQ: Social Communication Questionnaire; SF-36: 36-Item Short Form Health Survey; STAI: State-Trait

Anxiety Inventory; TAI: Trait Anxiety Inventory; VABS-II: Vineland Adaptive Behavior Scales - Second Edition; VBM: voxel-based morphometry; VLMT: Verbal Learning Memory Test.

Figure 4-1. PRISMA flow chart detailing the identification and selection of studies for review.



CHAPTER 5

fMRI AND MICROBIOME FINDINGS FROM THE CAN-BIND 12: EFFECTS OF PROBIOTICS ON SYMPTOMS OF DEPRESSION STUDY

5.1 Introduction:

Major Depressive Disorder (MDD) can be an incredibly debilitating disorder with an estimated lifetime prevalence of 5 - 17% worldwide (1) and contributes to a significant economic (2), personal, and societal burden (3). It is characterized in the Diagnostic and Statistical Manual, 5th edition (DSM-5) by persistent low mood or anhedonia, in addition to a variety of symptoms including feelings of guilt, low energy, suicidal thoughts, and difficulties associated with concentration, sleep, appetite, and psychomotor activity (4). MDD often coincides with many comorbidities such as anxiety (5), sleep (6), and metabolic disorders (7), and can meaningfully impact an individual's health and functioning. In addition to being highly prevalent and debilitating, MDD has proven to be difficult to treat in many cases. Traditional first-line treatments for depression, such as SSRIs, are estimated to have remission rates of around 30 – 45% (8). This suggests that more than half of individuals with MDD fail to remit after their first antidepressant treatment (9), and many are thought to be treatment resistant (10). In addition to many individuals failing to respond or remit after antidepressant treatment, there are significant factors (such as side effects, stigma, and cost) associated with these medications, that can serve as barriers to people seeking out or continuing with treatment (11). Research into alternative treatments is crucial for addressing these concerns and providing options to patients

and clinicians. Gut microbiome therapeutics, such probiotics and fecal microbiota transplantation, have recently emerged as research targets for potential alternative treatments.

Gut microbiome therapeutics include a range of treatments and supplements designed to influence the gut microbiome. The gut microbiome refers to the community of an estimated 10 - 100 trillion commensal bacteria that populate one's intestinal lumen (the gut microbiota) along with their genomes, microbial structural elements, metabolites, and environmental conditions (12). Recent investigations into the use of gut microbiome therapeutics for psychiatric populations have primarily focused on probiotics, fecal microbiota transplantation, and prebiotics. Probiotics are living bacterial species that are ingested by the recipient with the expectation of conferring a health benefit, potentially through affecting the balance of bacterial species in the gut microbiota (13). Fecal microbiota transplantation involves the collection and processing of stool typically from a healthy donor, which is then transplanted to the recipient, often through endoscopies, enemas, or freeze-dried and consumed orally. Prebiotics refer to any supplement or substance that is administered with the intention of effecting the gut microbiome that does not contain living bacteria such as, sugars, fibres, or minerals. There has been some evidence to support the efficacy of gut microbiome therapeutics in ameliorating depressive symptoms, particularly probiotics (14,15) and fecal microbiota transplantation (16), both as monotherapy and as adjuvant treatments (17). Despite some promising findings, research on gut microbiome therapeutics in psychiatry is still in the early stages, and far more research is needed before a consensus can be drawn and practices can be standardized across all studies, products, and clinical use.

The exact mechanisms by which gut microbiome therapeutics, and the gut microbiome can ameliorate psychiatric symptoms is still uncertain, but multiple pathways have been proposed.

These pathways make up the gut-brain-axis, allowing for the gut microbiome and central nervous system to communicate with, and affect, one another. The main pathways for the gut-brain-axis include communication via the nervous, immune, humoral and metabolic systems (18).

One example of how the gut microbiome can influence the rest of the body and the pathways of the gut-brain axis is through the interactions of microbial byproducts, such as short-chain fatty acids. Short-chain fatty acids are produced through the fermentation of non-digestible carbohydrates by gut microbiota and can affect and modulate many bodily systems, which in turn can affect each other (19). They influence the production and bioavailability of peripheral serotonin through interactions with enterochromaffin cells within the intestinal lining of the small and large intestine, which produce and excrete up to 95% of the body's serotonin (20). Though peripheral serotonin does not cross the blood-brain barrier, it can still influence the hypothalamic-pituitary-adrenal axis through interactions with corticotropin-releasing hormone and has been linked to anxiety-like behaviour in preclinical models. Short-chain fatty acids have also been found to be inherently anti-inflammatory, implying interactions with the immune system.

Though the aforementioned interactions may affect the brain indirectly through interactions with other systems, the gut microbiome and short-chain fatty acids can also have direct effects on the central nervous system. Short-chain fatty acids can directly innervate the nerves of the enteric nervous system and the vagus nerve, affecting key properties of the nervous system such as vagal tone (21). This has specific implications for psychiatry and mood disorders as there is evidence to suggest vagus nerve stimulation can be an effective treatment for affective disorders (22). Short-chain fatty acids are just one example of potential ways in which the gut microbiome may affect the body, and in turn, the central nervous system and psychiatric symptoms.

The structure and function of the central nervous system has always been a key area of research when investigating psychiatric illnesses, such as Major Depressive Disorder. Many treatments for MDD target the brain, with the intention to modulate the activity of specific regions, or concentrations of specific neurotransmitters. Common antidepressant treatments include medications designed to affect neurotransmitter concentrations such as selective serotonin reuptake inhibitors and monoamine oxidase inhibitors (23), and direct stimulation of the brain through treatments like electroconvulsive therapy or transcranial magnetic stimulation (24). Many of these treatments have been correlated with changes in functional connectivity between brain regions, with said changes sometimes being associated with treatment response (25–27). Functional connectivity is a measure of synchronicity in activity between regions of the brain. Resting state functional connectivity measures the correlation in activity between brain regions when subjects are at rest and allowing their minds to wander (28). When at rest, key neural networks that have been found to differ significantly between individuals with and without MDD, such as the default mode, salience, frontal parietal and affective networks, can be observed (29). Though many studies have investigated the effects of depression treatments on this resting-state functional connectivity, few have investigated the effects of gut microbiome therapeutics such as probiotics.

In this study, resting-state functional connectivity and microbiome data from the Canadian Biomarker Integration Network in Depression (CAN-BIND) 12: Effects of Probiotics on Symptoms of Depression (CBN12: EPSD) clinical trial was analysed. Differences in the change alpha diversity of the microbiome and resting state functional connectivity between key brain regions related to depression over the course of the trial were assessed in subjects from the placebo and probiotic groups. The aim of this analysis was to observe the effect of probiotic

consumption on microbiome composition and brain region functional connectivity in a population with Major Depressive Disorder. The functional connectivity analysis was designed to mirror the analysis conducted by Yamanbaeva et al. in 2023, which was the only analysis to date of resting-state functional connectivity changes associated with probiotics administration in a population with MDD (30). Yamanbaeva et al. observed two clusters, the right precuneus and left superior parietal lobule, with uniquely different changes in resting state functional connectivity with limbic structures between the probiotic and placebo groups. The hypotheses for this analysis was that probiotic consumption would lead to a significant increase in the alpha diversity of bacterial species collected from participants' stool samples, and a replication of Yamanbaeva et al.'s functional connectivity findings would be observed.

5.2 Methods

This is a secondary analysis of data from the CBN12: EPSD randomized controlled clinical trial (NCT03277586). The protocol for this trial has been published (31), with the primary analysis of the clinical, molecular, and preliminary microbial data being presented in the PhD thesis written by Dr. Caroline Wallace, but not yet published in a scientific journal. This analysis presents the analysis of alpha diversity from microbial data, and functional connectivity from the function magnetic resonance imaging (fMRI) data.

5.2.1 Study Design and Sample

The CBN12: EPSD study was a dual-phase 16-week double-blinded placebo-controlled randomized trial conducted through Providence Care Hospital and Queen's University in

Kingston, Ontario, Canada in partnership with Lallemand Health Solutions Inc. from 2019 – 2020. Full study procedures and details can be found in the published protocol (31). Adults ages 18 – 65 experiencing an ongoing major depressive episode and not currently taking any antidepressant medication or treatment were recruited from the Kingston community. MDD diagnosis as per DSM-IV criteria was confirmed using the Mini International Neuropsychiatric interview (32), and moderate depression defined as a score of 20 or greater on the Montgomery-Åsberg Depression Rating Scale (MADRS) (33) was required. Full inclusion/exclusion criteria can be found in the published protocol (31). Participants received either a probiotic supplement containing *Lactobacillus helveticus* R0052 (90%) and *Bifidobacterium longum* R0175 (10%) (CEREBIOME®, previously known as Probio’Stick®; Lallemand Health Solutions Inc., Montreal, Canada) or a placebo for 8 weeks. After the initial 8-week period, participants were assessed for response status with non-responders switching either from low-dose (6×10^9 CFU/day) to high dose (20×10^9 CFU/day) probiotic or from placebo to the low-dose probiotic respectively. The initial trial successfully recruited and enrolled 28 participants, 11 of which completed both baseline and week 8 fMRI scans and were included in functional connectivity analysis, and 12 with sufficient stool data to be included in microbiome analysis.

5.2.2 Measures

Clinical Depression symptoms were primarily assessed using the MADRS, a 10-item clinician rated scale used to measure and quantify the severity of various domains of depression symptoms (33). Response was defined as a 50% or greater reduction in MADRS score from baseline, with remission being a score of 10 or lower. MADRS interviews were conducted and scores recorded bi-weekly over the course of the study. Stool samples were collected at baseline, week 2, week 8 for microbiome composition analysis. Samples were collected in sterile

specimen containers and frozen at -80°C until analysis. Neuroimaging data was collected through 3-tesla Magnetic Resonance Imaging (MRI) scans conducted at baseline and week 8. Resting state fMRI data was recorded through a 10-minute whole-brain longitudinal relaxation time (T)₂ weighted Blood Oxygen Level Dependent (BOLD) Echo-Planar Imaging (EPI) series at a spatial resolution of $3.125 \times 3.125 \times 3$ mm and temporal resolution of 2 seconds during the awake resting state. Participants were instructed to relax but stay awake and allow their mind to wander while not thinking about anything in specific during the scan. A whole-brain (T)₁-weighted anatomical scan at 1mm^3 resolution was used as anatomical reference. Further details regarding the measures used in the clinical trial can be found in the published protocol (31).

5.2.3 Microbiome Composition Analysis

Stool samples were analysed in partnership with Lallemand Health Solutions Inc., using 16 Svedberg unit (S) ribosomal ribonucleic acid (rRNA) gene sequencing and their methodology. Bacterial DNA was extracted, and quantitative polymerase chain reaction (qPCR) was used to amplify the 16S hypervariable (V)₃-V₄ regions from all bacteria in each sample. DNA concentrations were determined using a Nanodrop. Samples were normalized and pooled for multiplexing using a liquid handling robot and loaded into an Illumina MiSeq for sequencing and clustered into operational taxonomic units (OTUs) based on sequence identity, then taxonomically classified using a common reference database. Resultant fastq files were exported for bioinformatics analyses. Relative abundance and diversity outcomes were produced from raw sequencing data using the bioinformatics platform Quantitative Insights Into Microbial Ecology 2 (QIIME 2). Alpha diversity was calculated to quantify the diversity of bacterial species observed in each sample. Four measures of alpha diversity were used in this analysis: (a) Faith's phylogenetic diversity (PD), (b) Pielou's evenness index, (c) Shannon's entropy, and (d)

observed operational taxonomic units (OTUs). Repeated-measures analysis of variances (RM ANOVAs) were used to compare the change in alpha diversity over time between the two groups. Mann Whitney-U tests were used to assess the difference between groups at baseline.

5.2.4 fMRI Analysis

Pre-processing: fMRI pre-processing was conducted using the Functional Magnetic Resonance Imaging of the Brain (FMRIB) Software Library (FSL), FMRI Expert Analysis Tool (FEAT) (34), with further motion correction and denoising through Independent Component Analysis based strategy for the Automatic Removal of Motion Artifacts (ICA-AROMA) (35) and Analysis of Functional NeuroImages (AFNI) (36,37) software. Brains were extracted from the fMRI scans using FSL's brain extraction tool (BET), with a fractional intensity threshold of 0.5 to remove the skull and neck, and the first 3 volumes were deleted. Noise was estimated through FSL, and motion corrected using FMRIB's Linear Image Registration Tool for Motion Correction (MCFLIRT) (38). Slice timing correction was interleaved, facial smoothing was set to 6, and intensity normalization was checked in order to be consistent with other CAN-BIND fMRI analyses. Scans were registered to T1-weighted anatomical scans, which were also BET extracted, with the Montreal Neurological Institute (MNI) 152 1mm T1 brain scan used as the standard space image (39). Boundary-based registration was used for the main structural image while 12 degrees of freedom was used for the standard space image. Nonlinear registration was used for the standard space image, and the warp resolution was set at 10 to be consistent with other CANBIND studies. ICA-AROMA was used to remove head motion artifacts, and band-passing and motion regression was conducted through AFNI using the `afni_proc.py` script and the motion regression file from ICA-AROMA.

ROI Selection, Masking, and Data Extraction: Regions of Interest (ROIs) were selected based on the ROIs used in the Yamanbaeva et al. paper with the intention of replicating their results (30). The ROIs included in the ROI-ROI analysis were the left and right orbitofrontal cortex (OFC), left and right insula, left and right hippocampus, left and right amygdala, left and right temporal poles, subcallosal cortex (subgenual anterior cingulate cortex (subgenual ACC)), and the two clusters identified by Yamanbaeva et al. in the right precuneus and left superior parietal lobule respectively. The clusters identified by Yamanbaeva et al. were found as a result of a seed-to-voxel analysis that they conducted. They used the limbic regions mentioned above as a large seed for their seed-to-voxel analysis and identified the clusters as having significant interactions with the seed.

ROIs were defined using version 1 of the automated anatomical labelling atlas 3 (AAL3v1) (40) with the medial, lateral, anterior and posterior OFC regions, left and right subgenual ACC regions, and middle and superior temporal pole regions being combined to create the left and right OFC, subcallosal cortex, and left and right temporal pole ROIs respectively. Each anatomical ROI mask was created using the `fslmaths` commands `-thr` and `-uthr` to isolate the masks from the AAL3v1 template. The masks for clusters in the precuneus and superior parietal lobules were created using `fslmaths` at the coordinates reported in the Yamanbaeva et al. paper ($x=16$ $y=-52$ $z=18$ and $x=-44$ $y=-42$ $z=50$ respectively) (30) with a kernel sphere of 5. The anatomical masks and cluster masks were then combined using `fslmaths`.

Individual participants' warp data was produced by FSL's FEAT was then inverted using the `invwarp--` command before being applied using the `applywarp--` command to the masks, warping the masks from standard space to native space for each participant. Correlation coefficients between each ROI were then produced using AFNI's 3dNetCorr. The fisher z scores

from the correlation tables were then vectorized through the MATrix LABoratory (MATLAB) (41) before being transferred to the Statistical Package for Social Sciences (SPSS) (42) for final analyses.

Functional Connectivity Analysis: Functional connectivity was analysed in SPSS using the fisher z transformed scores (rsFC) based on the correlation coefficients between each ROI. Independent samples t-tests were conducted between each ROI using the rsFC change score (week 8 – baseline) between each region to evaluate for significant treatment*time interactions. For ROIs with independent samples t-tests that approached significance, further independent samples t-tests were conducted using the baseline and week 8 scores separately to provide information regarding the differences between the probiotic and placebo group at each time point, and paired samples t-tests were conducted on the groups separately to evaluate the significance of the change in rsFC within each group. Independent samples t-tests on the change scores were first conducted focusing on the connections involving the two clusters identified by Yamanbaeva et al. with the intention of replicating their results. Afterwards, additional exploratory tests were run to identify potential differences in the rsFC between all 13 ROIs. The connections found to differ significantly were then correlated with change in the MADRS scores to see how changes in rsFC between these regions could be related to clinical symptom severity.

5.3 Results

5.3.1 Sample Characteristics

The target sample size for the CBN12: EPSD study was 108 participants, but the study was terminated early due to challenges with recruitment that were exasperated by the COVID-19

pandemic. The final sample size was 28 participants. Of the 28 participants recruited, 23 had at least one post-baseline measure for the primary outcome measure (MADRS) (probiotic n=13, placebo n=10). Of the 23 participants with at least one assessment post-baseline, 12 participants (probiotic n=7, placebo n=5) had stool samples at all time points from baseline to week 8, allowing them to be included in the alpha diversity analysis. 11 participants (probiotic n=6, placebo n=5), had baseline and week 8 MRI scans, making them eligible for the functional connectivity analysis. Baseline characteristics and demographics information for the participants included in the alpha diversity and functional connectivity analyses can be found in Table 6-1 and Table 6-2 respectively. Mann-Whitney U tests were performed to check for significant differences between the placebo and probiotic groups on age, baseline MADRS scores, and change in MADRS score from baseline to week 8. The groups did not differ significantly on baseline or change in MADRS scores (alpha diversity baseline MADRS $U=10.50$, $p=0.2668$ and change score $U=14.50$, $p=0.639$; functional connectivity baseline MADRS $U=10.00$, $p=0.429$ and change score $U=14.50$, $p=0.931$). The alpha diversity sample differed significantly in age between groups ($U=5.50$, $p=0.048$), while the functional connectivity sample did not ($U=5.50$, $p=0.082$). χ^2 tests confirmed sex differences were not significant between groups for either analysis, using the Fisher's exact test as the minimum expected count assumption was violated (alpha diversity Fisher's exact test $p=0.636$, functional connectivity Fisher's exact test $p=0.545$).

5.3.2 Alpha Diversity

Kolmogrov-Smirnov and Shapiro-Wilk tests were non-significant for all alpha diversity measures at all time points, except for week 2 Shannon's entropy scores, suggesting the data was normally distributed and the distributions did not differ significantly between groups. Baseline

Mann-Whitney U tests were non-significant for all alpha diversity measures except for observed OTUs ($U=5.00$, $p=0.048$) suggesting a significant difference in baseline observed OTUs between groups, which was confirmed through an independent t-test ($T(10)=-2.725$, $p=0.021$). Overall RM ANOVAs between-subjects were non-significant except for observed OTUs ($F(1,9)=5.527$, $p=0.043$), which was likely due to the significant differences between groups at baseline.

Pairwise comparisons found a significant increase in Shannon's entropy index from week 2 to week 8 in probiotic group ($M=0.451$, $SD=0.185$, $p=0.037$) (Figure 6-1C). In addition to the significant increase in Shannon's entropy index, all measures of alpha diversity in the probiotic group showed a trend of decreasing from baseline to week 2, and increasing from week 2 to week 8 (Figures 6-1A – 6-1D). It is possible a larger sample size would make these trends significant. No measures of alpha diversity at any time point were significantly correlated to MADRS scores at week 8, week 16, or the change in MADRS scores from baseline to week 8.

5.3.3 Functional Connectivity Analysis

The ROI-ROI analysis of functional connectivity between the aforementioned ROIs revealed significant differences in the change in rsFC between some regions in the probiotic group compared to the placebo group involving the precuneus cluster, orbitofrontal cortex (OFC), hippocampus, superior parietal lobule cluster, insula, amygdala, and temporal poles.

Precuneus Cluster – Left Orbitofrontal Cortex: The precuneus cluster identified by Yamanbaeva et al. had significant differences between groups in the change in rsFC between that cluster and the orbitofrontal cortex (OFC) and hippocampus. An independent samples t-test of the change in rsFC between the precuneus cluster and left OFC showed that the probiotic group increased ($M=0.211$, $SD=0.290$) whereas the placebo group decreased ($M=-0.126$, $SD=0.185$) resulting in a nearly significant difference between the change in rsFC between the two groups

($t(9)=-2.24$, $p=0.052$) (Figure 5-2A). Independent samples t-tests found no significant differences between the probiotic ($M=-0.273$, $SD=0.248$) and placebo ($M=-0.086$, $SD=0.192$) groups at baseline ($t(9)=1.38$, $p=0.202$), but significant differences at week 8 (probiotic $M=-0.062$, $SD=0.113$; placebo $M=-0.211$, $SD=0.084$; $t(9)=-2.44$, $p=0.037$). Paired samples t-tests showed the changes were not significant within either group (probiotic $M=0.211$, $SD=0.290$, $t(5)=-1.79$, $p=0.134$; placebo $M=0.126$, $SD=0.185$, $t(4)=1.52$, $p=0.204$).

Precuneus Cluster – Right Hippocampus: The independent samples t-test of the change in rsFC between the precuneus cluster and right hippocampus found the decrease in the probiotic group ($M=-0.130$, $SD=0.120$) to differ significantly from the increase observed in the placebo group ($M=0.109$, $SD=0.157$) ($t(9)=2.87$, $p=0.019$) (Figure 5-2B). Independent samples t-tests at baseline (probiotic $M=0.271$, $SD=0.164$; placebo $M=0.141$, $SD=0.128$) and week 8 (probiotic $M=0.142$, $SD=0.152$; placebo $M=0.250$, $SD=0.154$) were not significant ($t(9)=-1.45$, $p=0.18$; $t(9)=1.18$, $p=0.27$). Paired samples t-tests showed a significant decrease in the probiotic group ($M=-0.130$, $SD=0.120$, $t(5)=-2.65$, $p=0.023$), but no significant change in the placebo group ($M=0.109$, $SD=0.157$, $t(4)=1.55$, $p=0.098$).

Superior Parietal Lobule Cluster – Left Insula: The cluster in the superior parietal lobule identified by Yamanbaeva et al. showed significant differences in the change in rsFC with the left insula between groups. An independent samples t-test of the change in rsFC showed the decrease in the probiotic group ($M=-0.850$, $SD=0.189$) to approach a significant difference from the increase observed in the placebo group ($M=0.198$, $SD=0.255$) ($t(9)=2.12$, $p=0.063$) (Figure 5-2C). Independent samples t-tests showed that the groups differed significantly at baseline (probiotic $M=0.210$, $SD=0.123$; placebo $M=-0.073$, $SD=0.174$; $t(9)=-3.157$, $p=0.012$), and became similar by week 8 (probiotic $M=0.125$, $SD=0.132$; placebo $M=0.126$, $SD=0.155$;

$t(9)=0.013$, $p=0.99$). Paired samples t-tests for each group individually revealed neither change to be significant within each group (probiotic $M=-0.850$, $SD=0.189$, $t(5)=-1.10$, $p=0.32$; placebo $M=0.198$, $SD=0.255$; $t(4)=1.74$, $p=0.16$).

Left Temporal Pole – Right Temporal Pole: The temporal poles also displayed significant differences in their change in rsFC with each other over time between the two treatment groups. An independent samples t-test using the change in rsFC between the left and right temporal poles showed the decrease observed in the probiotic group ($M=-0.039$, $SD=0.198$) to differ significantly from the increase observed in the placebo group ($M=0.220$, $SD=0.157$) ($t(9)=2.364$, $p=0.042$) (Figure 5-2D). Independent samples t-tests at baseline and week 8 revealed no significant differences between the probiotic (baseline $M=0.601$, $SD=0.252$; week 8 $M=0.562$, $SD=0.378$) and placebo (baseline $M=0.510$, $SD=0.335$; week 8 $M=0.730$, $SD=0.286$) groups (baseline $t(9)=-0.516$, $p=0.62$; week 8 $t(9)=0.813$, $p=0.44$). Paired samples t-tests showed the increase in the placebo group to be significant ($M=0.220$, $SD=0.157$, $t(4)=3.134$, $p=0.035$), while the decrease in the probiotic group was not ($M=-0.039$, $SD=0.198$, $t(5)=0.478$, $p=0.65$).

Right Hippocampus – Left Amygdala: The change in rsFC between the right hippocampus and left amygdala was found to be significantly different between the two groups. An independent samples t-test found the slight increase in the probiotic group ($M=0.157$, $SD=0.328$) to approach a significant difference from the decrease observed in the placebo group ($M=-0.232$, $SD=0.272$) ($t(9)=-2.11$, $p=0.064$) (Figure 5-2E). Independent samples t-tests at baseline and week 8 revealed the groups to be nearly significantly different at baseline (probiotic $M=0.209$, $SD=0.151$; placebo $M=0.514$, $SD=0.299$; $t(9)=2.20$, $p=0.055$), and not significantly different at week 8 (probiotic $M=0.367$, $SD=0.252$; placebo $M=0.282$, $SD=0.131$; $t(9)=-0.67$, $p=0.52$). Paired samples t-tests for each group individually revealed neither change to be

significant with each group (probiotic $M=0.157$, $SD=0.328$, $t(5)=1.18$, $p=0.29$; placebo $M=-0.232$, $SD=0.272$, $t(4)=-1.91$, $p=0.129$).

rsFC Change Correlations to MADRS Change: The five connections found to have a significant or nearly significant difference in the change in rsFC between the placebo and probiotic groups were then correlated with the change in MADRS scores from baseline to week 8 and baseline to week 16. No correlations were found to be significant.

5.4 Discussion

5.4.1 Alpha Diversity

This analysis showed mixed results when considering the effect of probiotic intervention on the alpha diversity of bacterial species observed in the stool samples. Shannon's entropy index was the only measure of alpha diversity that was observed to have a significant increase in the probiotic group, while there was no significant changes in the placebo group. The four measures of alpha diversity are all designed to measure the diversity of bacterial species in a sample, but do so in different ways (43).

Observed OTUs is a count of the different operational taxonomical units within a sample. This can provide insight into the number of different bacterial species in the sample but does not provide insight into the relative abundance of each species, or consider how closely or distantly related the bacterial species are. Pielou's evenness index measures the evenness of the distribution of species within a sample, while Shannon's entropy index estimates the likelihood of randomly sampling different species from a sample by incorporating the evenness and richness of each species. Finally, Faith's PD measures the diversity of the sample by calculating

the sum of branch lengths between the observed species on a phylogenetic tree. An example of how the different measures can provide different insights into a population would be if there was a sample with multiple different bacterial species, all from the same genus, and with one species being far more abundant than the others. The observed OTUs would simply count the number of different species, while the Shannon's entropy index and Pielou's evenness index would be relatively low since one species is dominating the sample, and the Faith's PD would also be relatively low since all species belonged to the same genus.

The findings from this study, where the only significant change in alpha diversity measures between the two groups was a significant increase in Shannon's entropy index in the probiotic group from week 2 to week 8, would suggest that the probiotic intervention may have affected the distribution of certain bacterial species, skewing the population towards rarer species and increasing the entropy of the sample, while resisting the introduction of new bacterial species, and thus not greatly affecting the observed OTUs, Faith's PD, or Pielou's evenness scores. This would suggest the probiotic intervention may have had a stabilising effect on the gut microbiota. That being said, there was a trend for all measures of alpha diversity to decrease from baseline to week 2 in the probiotic group, and then increase from week 2 to week 8. This trend could become significant with greater sample sizes, and would suggest the probiotic may take around two weeks to begin increasing the alpha diversity of the gut microbiome. This could be due to time needed for the bacteria introduced by the probiotic to begin colonizing the intestinal lumen, or perhaps competition between the newly introduced and preexisting bacteria could drive the initial decrease in alpha diversity before eventually flourishing and increasing alpha diversity.

The literature on the effects of probiotic administration on alpha diversity in individuals with MDD is scarce, inconsistent and often contradictory (44), making it difficult to determine how these findings fit into the broader landscape of research in this area. Many studies have found no significant differences between placebo and probiotic groups after intervention, while others have found probiotics to have a maintenance effect as opposed to a decrease observed in placebo groups (45,46). This may be due to a variety of factors including the variation in probiotic formulations between studies, insufficient sample sizes, or the potential changes in dietary and lifestyle habits of participants in the relatively short durations of most studies. The limitations of the data used in this analysis including the small sample size, significant difference between observed OTUs at baseline between the two groups, and the lack of correlations with dietary data make it difficult to draw any meaningful conclusions from these findings.

5.4.2 Functional Connectivity

To my knowledge, this is the first analysis of the changes in rsFC in a population with depression receiving solely probiotic or placebo intervention. The Yamanbaeva et al. analysis was similar and used as a template for this analysis but included a population receiving probiotic intervention as an add-on to treatment as usual with traditional antidepressants. This analysis provides unique insight into the potential independent effects of probiotics and gut microbiome manipulation on the resting state functional connectivity of individuals with MDD. The findings from the functional connectivity analyses suggest that probiotic intervention may affect the connection and co-activity of specific brain regions at rest in individuals with depression in a manner unique to this intervention as compared to placebo.

Precuneus: The cluster in the right precuneus was found to have significant or near significant differences in the change in rsFC over time between the probiotic and placebo groups with two regions, the left OFC and the right hippocampus. The precuneus is part of the default mode network (DMN) (47), which is active at rest and has been associated with spontaneous self-referential thought (48). The precuneus is specifically thought to support processes within the DMN related to episodic memory and theory of mind (49). In the context of MDD, DMN connectivity has been found to be altered in comparison to healthy controls and associated with rumination and negative self-thought (48).

This analysis found rsFC connectivity between the right precuneus cluster and left OFC to increase in the probiotic group and decrease in the placebo group. Though this change in rsFC was not found to be significantly correlated with depression symptoms severity in this sample, other literature would suggest that this effect may have been counterproductive to the ameliorative effects of probiotic supplementation. A large-scale study and analysis of the functional connectivity of the precuneus in medicated and unmedicated individuals with depression conducted by Cheng et al. found unmedicated patients to have higher rsFC between the precuneus and OFC in comparison to healthy controls, with traditional antidepressant medication reducing the rsFC between the two regions (50). This would suggest that the increase in rsFC observed in the probiotic group may have contributed negatively to their depressive symptoms. It is theorized that the OFC is involved with non-reward/punishment systems, so increased functional connectivity with the precuneus which is involved with the self-referential and ruminative systems like the DMN, could contribute to the increased sensitivity to non-reward events, and self-deprecating or overly punitive characteristics of depression (50,51).

This analysis found the rsFC between the precuneus cluster and right hippocampus to decrease in the probiotic group and increase in the placebo group. The decrease in the probiotic group was significant within the group in addition to the significantly different change observed between groups. Though this change was also not found to be significantly correlated with changes in MADRS scores, in the context of the rsFC analysis conducted by Cheng et al., these findings once more have negative implications for the effects of probiotics on symptoms of depression. Cheng et al. found the hippocampus to be the only region where rsFC with the precuneus was reduced in non-medicated patients with depression compared to healthy controls and increased to the levels of healthy controls in medicated patients. The significant decrease related to probiotic intervention found in this study would theoretically worsen this observed difference between unmedicated patients and medicated patients or healthy controls. Higher baseline rsFC between the precuneus and hippocampus has also been associated with earlier response to antidepressant treatment (52). The hippocampus is thought to be involved with episodic memory, with the right hippocampus predominantly being associated with visuospatial memory (53). Since the precuneus is also associated with episodic memory and sense of self (54), a reduction in the functional connectivity between the two regions could contribute negatively to the cognitive symptoms associated with depression.

Superior Parietal Lobule - Insula: As for the findings from the analysis of the superior parietal lobule cluster, the only significantly different change in rsFC between the two groups was with the left insula. Further analysis revealed the groups differed significantly at baseline and became similar at week 8, with the neither change being significant within each group. The probiotic group remained relatively stable, with a slight decrease, and the placebo group increased but not by a statistically significant amount when looking at the group separately. This

change in rsFC was not significantly correlated with depressive symptoms. The superior parietal lobule is thought to be involved in cognition and working memory and is part of the DMN that has displayed hyperconnectivity in depression (29,55,56). The insula has been associated with many roles with clear implications for depression such as feelings, empathy, and processing uncertainty in decision making (57). Few studies have found associations between rsFC of the superior parietal lobule and insula with depression features, symptom severity, or treatment response, but a reduction in rsFC between the right superior parietal lobule and right dorsal angular insula has been associated with repetitive negative thinking (58). This could suggest that the relative decrease in the rsFC of the probiotic group could be detrimental when it comes to repetitive negative thinking in depression, but the significant baseline differences, small sample size, and other limitations of this analysis make it hard to draw any meaningful conclusions.

Left Temporal Pole – Right Temporal Pole: The change in rsFC between the right and left temporal poles was found to differ significantly between the probiotic and placebo groups. The placebo group was shown to have a significant increase in the rsFC between the poles, while the probiotic had a slight, insignificant decrease. The temporal poles are involved with assessing emotional significance through the integration of contextual cues from the environment (61). Increased temporal pole rsFC with the amygdala has been observed in MDD (61), but there is little evidence to suggest the rsFC between the two poles is relevant to MDD. The left temporal pole is theorized to be a semantic hub, linking other regions involved with semantic content (ie. object names and features) such as the posterior and anterior temporal lobes (62,63), while the right temporal pole is thought to be involved with emotion and socially relevant memory (64). Based on their differences in functioning, an increase in co-activity at rest in the context of MDD could suggest rumination around emotional and social memories attached to certain objects, but

further research would be needed to confirm this. The significant increase in the placebo group compared to the relative stability observed in the probiotic group could have implications regarding the effects of probiotics on brain functioning related to MDD, but this change in rsFC was not found to be significantly correlated with depressive symptoms in this study.

Hippocampus – Amygdala: The final regions with significant differences in the change in rsFC between the probiotic and placebo groups were the right hippocampus and left amygdala. Further analysis revealed the groups nearly differed significantly at baseline before becoming similar by week 8, with the rsFC in the probiotic group increasing and decreasing in the placebo group. The change in this rsFC was not found to be significantly correlated with change in MADRS scores. The literature seems to suggest age may play a role in regards to the effect of depression on this rsFC as Cullen et al. observed hypoconnectivity between the amygdala and left hippocampus in adolescents (59), while Tang et al. observed hyperconnectivity between the amygdala and right hippocampus in adults but not adolescent (60). Lower rsFC between the two regions has also been associated with increased depression severity (59). These findings suggest the probiotic intervention had a beneficial effect, by increasing the rsFC between the right hippocampus and left amygdala, as opposed to the decrease observed in the placebo group, and this may be especially salient to adolescent patients. That being said, the significant differences between groups, and lack of significant change within groups, in addition to the limitations of this study, make it hard to confirm the validity of this finding.

Comparison with Yamanbaeva et al.: When comparing the functional connectivity from the two clusters identified by Yamanbaeva et al. with the findings of this analysis, the only time*group interaction that was significant in both analyses was the precuneus cluster with the right hippocampus, however there was an opposite direction of the change in rsFC. Yamanbaeva

et al. observed the rsFC between the precuneus cluster and right hippocampus to increase in the probiotic group and decrease in the placebo group (30), whereas this analysis observed a decrease in the probiotic group and increase in the placebo group. The differences in rsFC observations between the two studies could be attributed to the many differences in study design and interventions. The participants in Yamanbaeva et al.'s analysis were receiving probiotic supplementation in addition to the treatment as usual (TAU). All of their participants were receiving antidepressant medication, which likely affected rsFC. This effect may have been mitigated considering both the placebo and probiotic groups included in their analysis were receiving TAU so the difference in change in rsFC between the two groups could be attributed only to probiotic intervention, but there could have been an interaction between the antidepressants and probiotics, or differences in the specific antidepressants used between groups, which affected the change in rsFC. In addition to this, the probiotic used by Yamanbaeva et al. differed greatly from the probiotic used in this study. This is a limitation that affects all probiotic and gut microbiome therapeutic research, as the variation in probiotic composition and dosages makes it difficult to compare findings. The probiotic used by Yamanbaeva et al. included six bacterial species in addition to the two that comprised the probiotic used in this study. The percentage of the strains comprising their product was not reported, so it is unknown how similar the composition of the two probiotics is. The participants analysed by Yamanbaeva et al. received a dose of 900 billion CFU/day, in comparison to the 6 – 20 billion CFU/day used in this study. The other significant difference between the two analyses is the sample sizes of each group. Yamanbaeva et al. had 14 and 18 participants in the probiotic and placebo groups respectively, as opposed to the 6 and 5 participants in the respective groups for this analysis. With these significant differences and limitations in mind, it is even more interesting that significant

differences in the change in the rsFC of the precuneus and superior parietal lobule clusters with other brain regions between the probiotic and placebo groups were observed in both studies. This analysis provides more evidence for the relevance of these two clusters in addition to the various prefrontal and limbic regions when it comes to the neurobiological effects of probiotics in a population with MDD.

5.4.3 Limitations

The greatest limitation of this analysis is the small sample size. With a total of 11 participants for the fMRI, and 12 for the alpha diversity analysis, it is very difficult to know the validity and replicability of these findings. The clinical trial was terminated early due to recruitment difficulties exasperated by the COVID-19 pandemic and thus was significantly underpowered for the primary analysis of the clinical effect of probiotic intervention, and also underpowered for these analyses. This population did not display a significant difference clinically between the change in depressive symptoms observed in the probiotic and placebo groups, which in turn make correlations between rsFC changes and response likelihood or symptom improvement difficult. It is possible that the findings regarding both rsFC changes alone and rsFC changes in relation to symptom improvement would be different with a larger sample size.

Another major limitation to the generalisability of these findings, and probiotic studies in general, is the specific nature of the investigational product and intervention. This study used a two-strain probiotic developed by Lallemand Health solutions, with participants in the probiotic group receiving 6 billion CFU/day initially, and non-responders increasing to 20 billion CFU/day

at week 8. The specific composition and dose of this intervention is unlikely to be repeated in future clinical trials, making it difficult to directly compare results. Unlike traditional antidepressants such as citalopram, with a consistent chemical makeup and a consensus on dose ranges, probiotics vary greatly from one to another in both composition and dosages. This is a limitation that is unlikely to be overcome until there have been a sufficient number of clinical trials to determine best practices for probiotic use in MDD treatment and research.

5.5 Conclusions

The findings from these analyses suggest the probiotic intervention alone may not be effective in eliciting the resting state functional connectivity changes traditionally associated with antidepressant response or depression symptom improvement, with the exception of the rsFC between the right hippocampus and left amygdala and likely does not greatly affect the alpha diversity of the gut microbiota, but may facilitate the proliferation of rarer bacterial species. The ameliorative effects of probiotic intervention on symptoms of depression has been repeatedly observed (14,65), but was not observed in this specific trial. These findings suggest that the mechanisms by which a beneficial effect of probiotics can occur may not be through changes in functional connectivity between brain regions at rest. The observation of these differences in rsFC changes with such a small sample size could point to even larger differences that could be revealed through the analysis of larger samples. This was a novel investigation into the effects of probiotic monotherapy on symptoms of depression and can be used as a stepping stone and foundation for larger analyses. That being said, there are many limitations that affect the generalisability of these findings, and further research with larger sample sizes is required to draw a significant conclusion.

5.6 References

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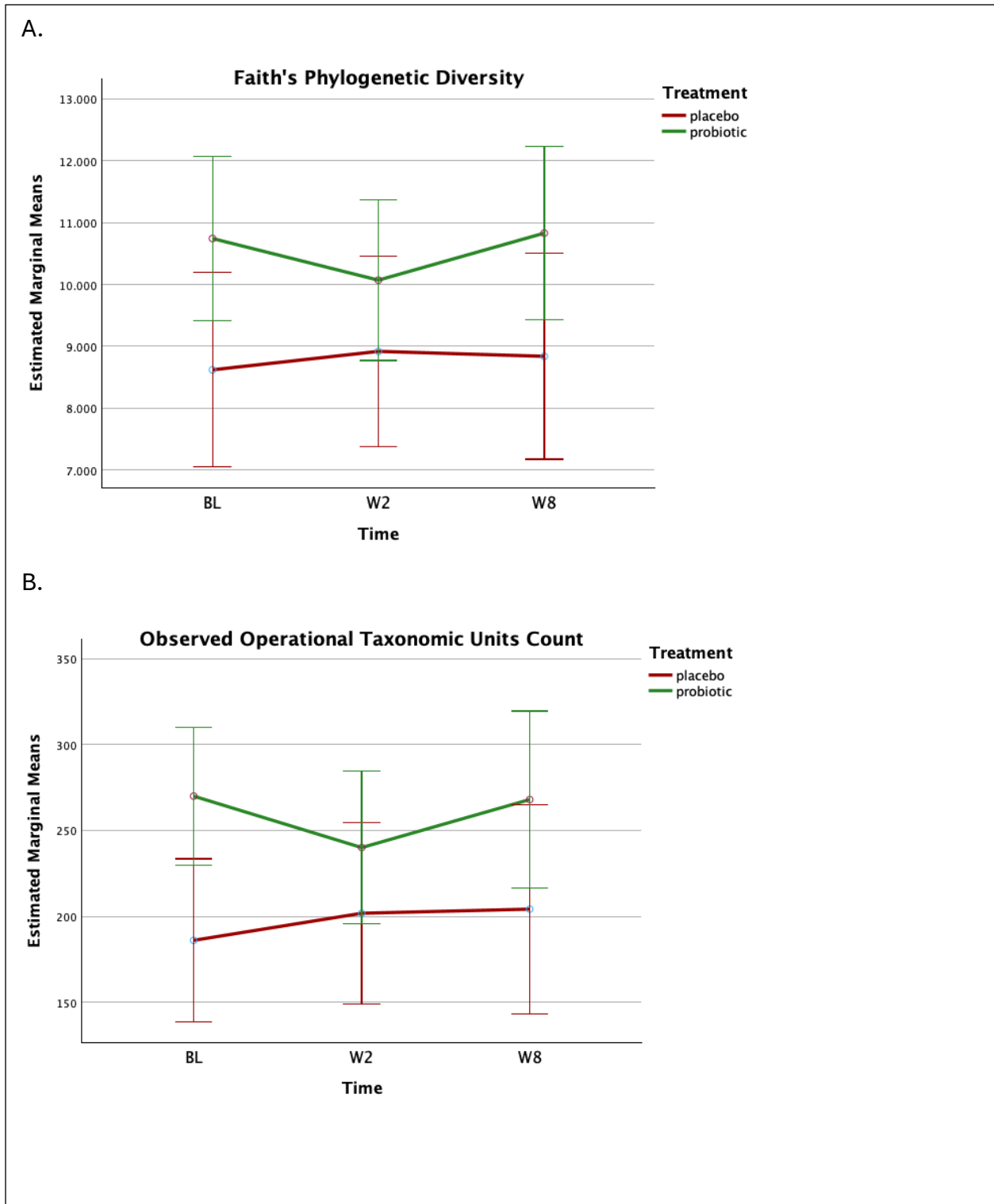
Table 5-1. Alpha diversity sample characteristics and demographics information.

Alpha Diversity Analysis	Probiotics (n=7)	Placebo (n=5)	Between-Group Comparisons
Demographics			
Age (years), mean (SD)	35.29 (11.80)	23.20 (6.30)	$U=5.50, p=0.048$
Sex Male/Female	2/5	1/4	Fisher's exact test $p=0.636$
MADRS			
Baseline, sum score, mean (SD)	28.14 (5.24)	25.80 (2.17)	$U=10.50, p=0.268$
Change score: Week 8 - Baseline, mean (SD)	-7.86 (-6.62)	-8.60 (-6.03)	$U=14.50, p=0.639$

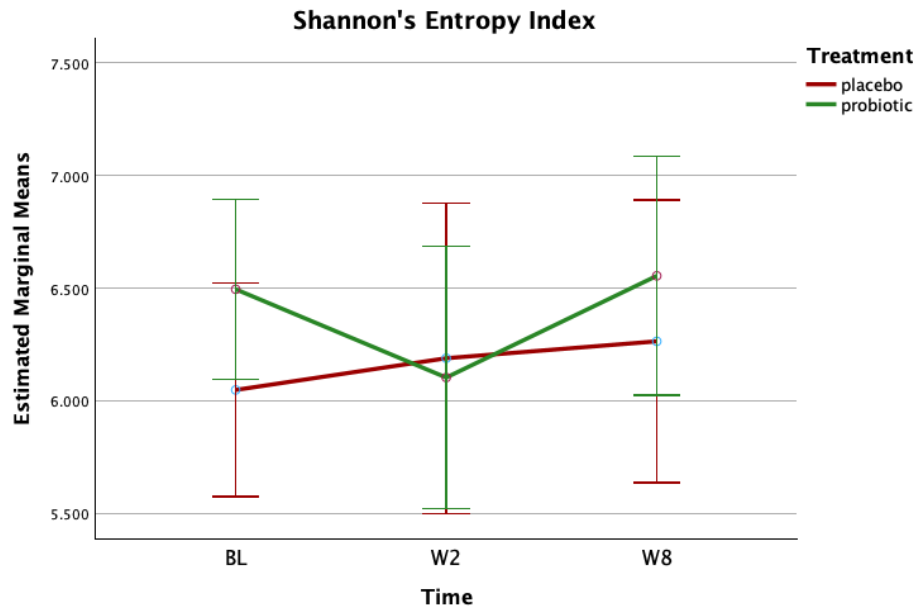
Table 5-2. Functional connectivity analysis sample characteristics and demographics information.

Functional Connectivity Analysis	Probiotics (n=6)	Placebo (n=5)	Between-Group Comparisons
Demographics			
Age (years), mean (SD)	31.33 (9.50)	23.00 (6.48)	$U=5.50, p=0.082$
Sex Male/Female	1/5	2/3	Fisher's exact test $p=0.545$
MADRS			
Baseline, sum score, mean (SD)	28.12 (5.74)	25.20 (2.17)	$U=10.00, p=0.429$
Change score: Week 8 - Baseline, mean (SD)	-7.33 (-6.35)	-7.20 (-5.93)	$U=14.50, p=0.931$

Figure 5-1. Changes in alpha diversity over three time points (baseline, week 2, and week 8) as measured through A) Faith's phylogenetic diversity, B) observed operational taxonomic units count, C) Shannon's entropy index, and D) Pielou's evenness index.



C.



D.

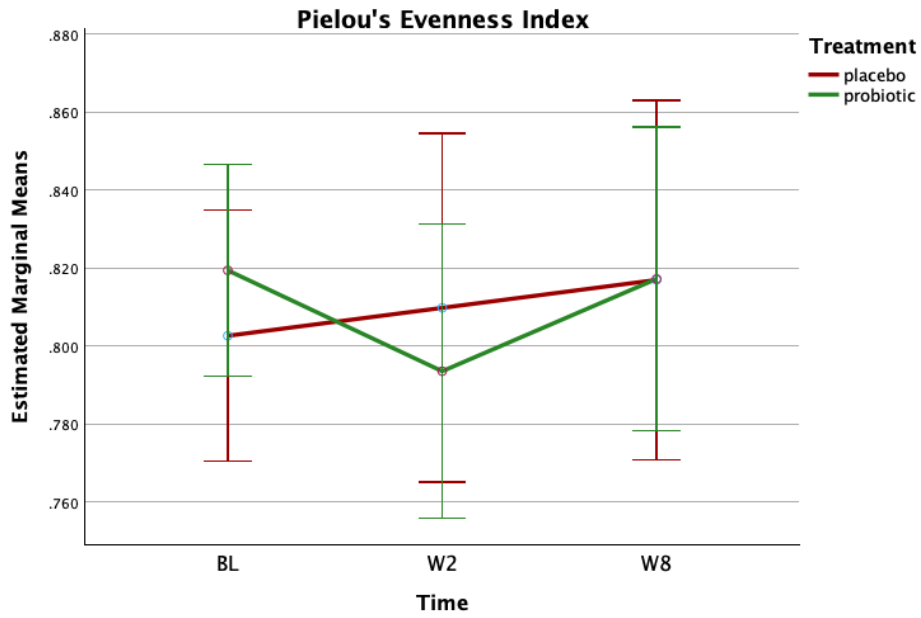
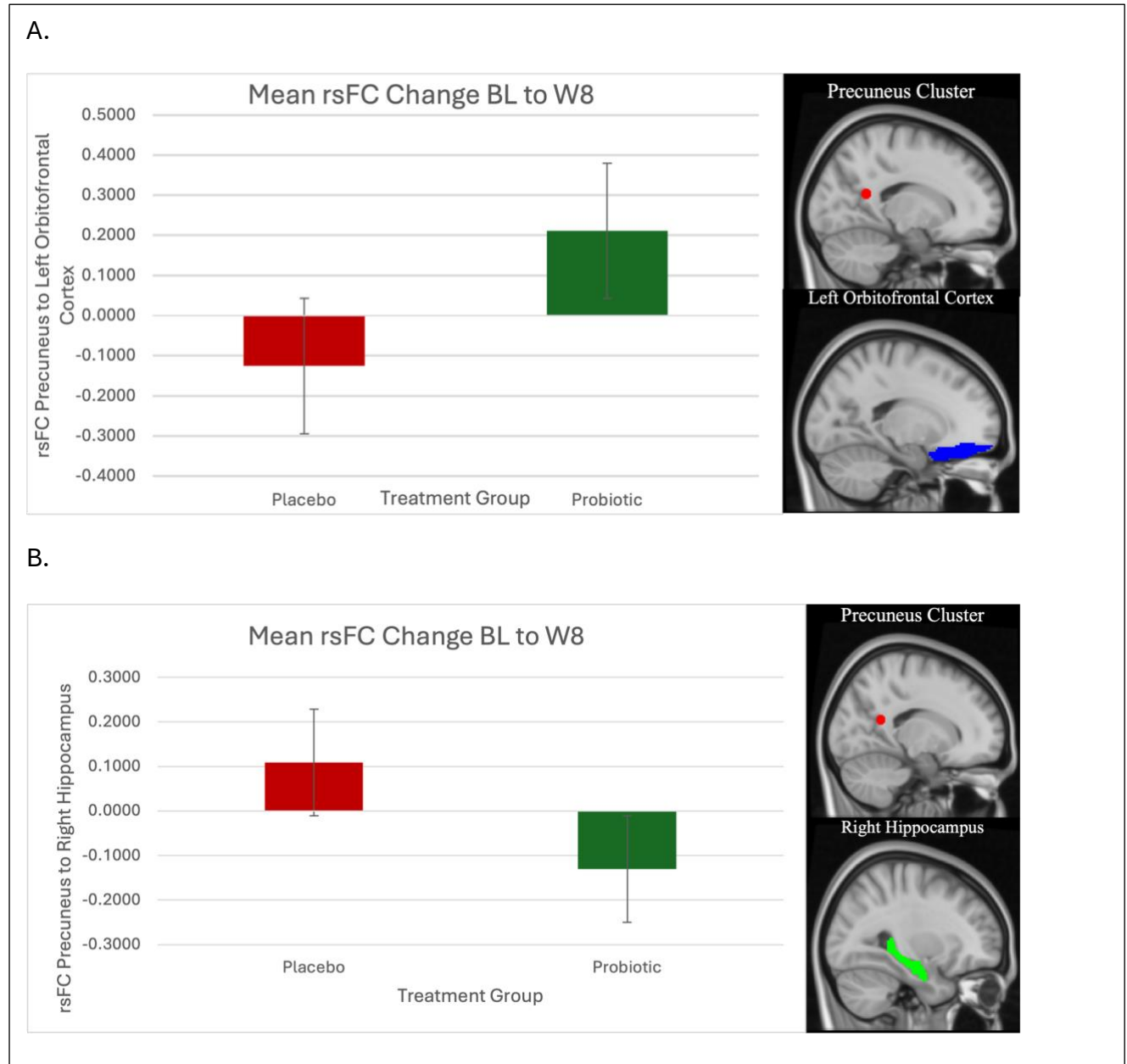
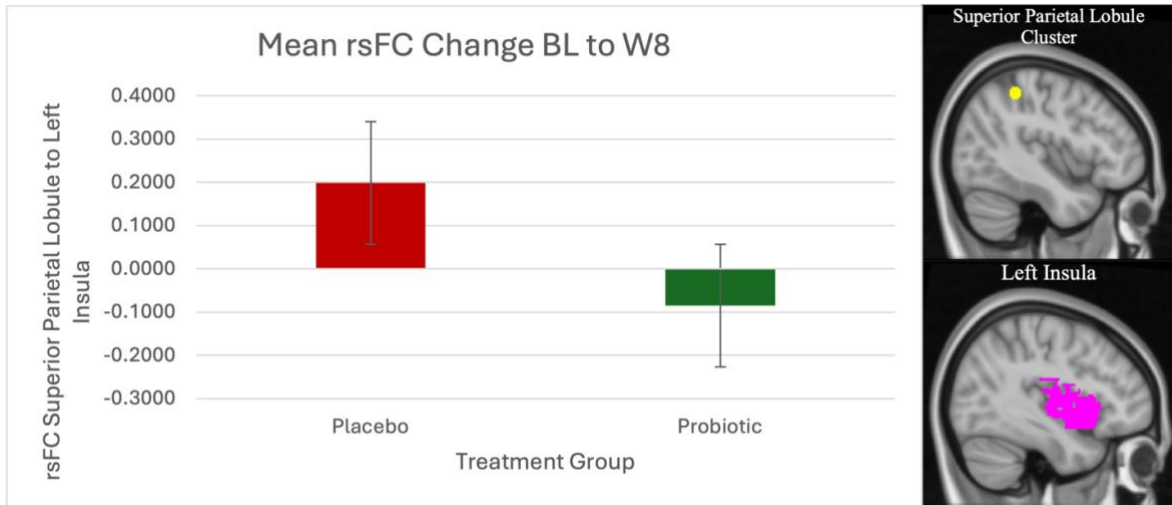


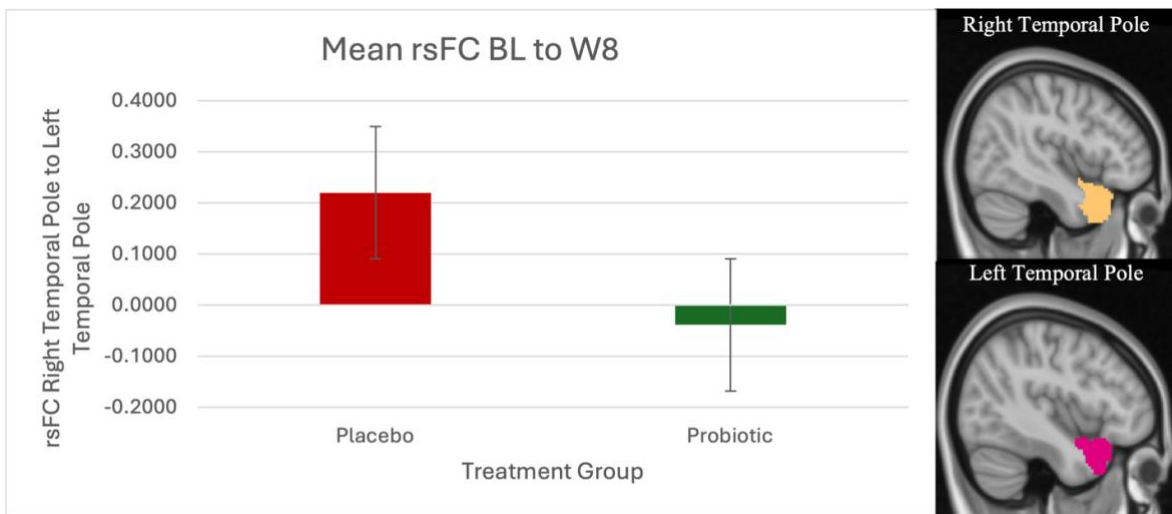
Figure 5-2. Mean change in resting state functional connectivity (rsFC) from baseline (BL) to week 8 (W8) between the A) precuneus cluster and left orbitofrontal cortex (OFC), B) precuneus cluster and right hippocampus, C) superior parietal lobule and left insula, D) right temporal pole and left temporal pole, and E) right hippocampus and left amygdala.



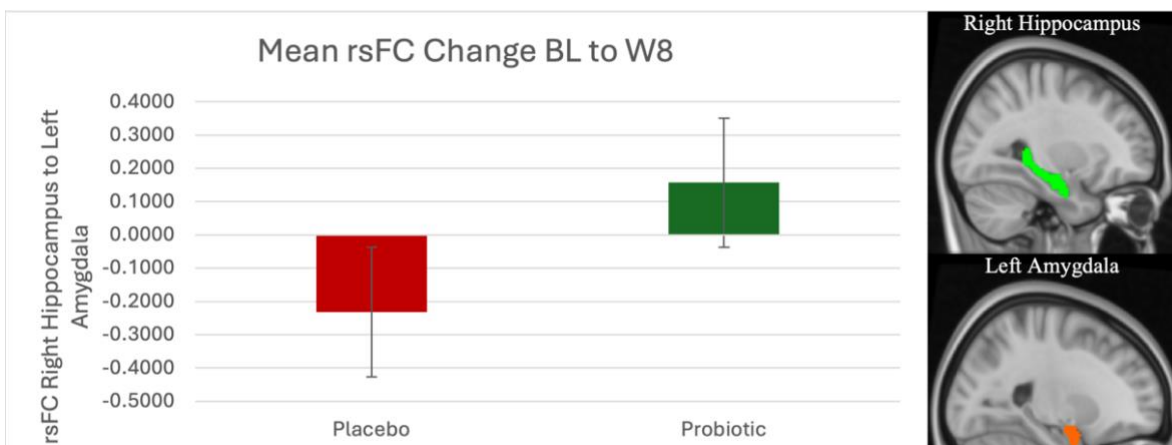
C.



D.



E.



CHAPTER 6

DISCUSSION AND CONCLUSIONS

6.1 Prologue

In this concluding chapter of my thesis, I will provide a comprehensive overview of the primary and secondary research included in previous chapters, explore the potential implications of the findings, discuss the limitations of the research, and identify areas to consider for future investigations. The primary aim of this section is to consolidate the findings discussed in previous chapters and interpret them within the broader context of the field.

6.2 Summary

In chapter 1, the findings of clinical trials investigating the use of probiotics, prebiotics and synbiotics in psychiatry, and the proposed mechanisms of action for said gut therapeutics were reviewed and discussed. This literature review served as an introduction to the world of microbiome modulation in the field of psychiatry and outlined the current research landscape. Through this review, it was shown that the field has primarily focused on the efficacy, safety, and tolerability of probiotics used in the context of Major Depressive Disorder (MDD), with far fewer studies investigating their effects on anxiety and schizophrenia symptoms, and some collecting immune, humoral, and oxidative stress biomarkers. Given the nascency of this field of research and the high prevalence and burden associated with MDD (1–3), it is reasonable for this to be the focus of the majority of research, but significant gaps in our understanding of microbiome manipulation in psychiatry, particularly in regards to other illnesses and the mechanisms of action of these treatments, remain.

Chapter 1 discussed the evidence supporting the efficacy of probiotic monotherapy in reducing depressive symptoms in individuals with MDD, and stress and anxiety in healthy or moderately stressed individuals, while highlighting the uncertainty of these findings due to the limited number and significant limitations of these studies. The chapter introduces some findings from studies investigating the use of probiotics in combination with other treatments, which are then expounded upon in the following chapter, and concludes with a summary of biomarker findings, potential mechanisms of action, and the limitations seen across the field. The lack of studies investigating and reporting physiological changes associated with probiotic intervention and the ambiguity of the characteristics and importance of the many proposed mechanisms of action, foregrounds the need for physiological analyses of the effects of gut microbiome therapeutics in psychiatry, in addition to efficacy, safety, and tolerability studies.

Chapter 2 continues to characterise the current research landscape by conducting a systematic review of studies investigating probiotic intervention administered in conjunction with traditional treatments for psychiatric disorders (4). As is consistent with research in this field, the majority of studies investigated the effects of adjuvant probiotic treatment on MDD, while one study investigated the effects on generalised anxiety disorder (GAD) (5), and two studies focused on schizophrenia (6,7). Of the five studies in populations with MDD, four observed a greater reduction in depressive symptoms in the adjuvant probiotic group when compared to the monotherapy group (8–11). The remaining study did not find a significant effect on depressive symptoms but observed a positive correlation between probiotic administration and cognitive performance (12). The sole GAD study also observed improved clinical outcomes in the adjuvant group as opposed to the sertraline monotherapy group (5). In contrast, both schizophrenia studies did not observe any significant differences in clinical outcome measures

between groups. However, their findings suggested adjuvant probiotic administration may have improved the tolerability of the antipsychotic treatments (6,7). The systematic review then went on to describe potential mechanisms for the interactions between probiotics and psychotropic medications, with a focus on explanations for the changes in biological and physiological features reported in the studies in relation to the current understanding of the gut-brain axis (GBA) and probiotic mechanisms of action. Considering the evidence suggesting the benefit of combining gut microbiome therapeutics with traditional psychotropic medication, an understanding of the interactions between treatments is critical.

Chapter 3 then expands the scope of gut microbiome therapeutics to include fecal microbiota transplantation (FMT). As opposed to the introduction of specific bacterial species through probiotics, or the enrichment of the environment of the gut microbiota through prebiotics, FMT allows for the transplantation of a healthier fecal microbiota into a recipient. This transplantation trades off precise microbiome manipulation in favor for effecting a drastic and immediate change in one's microbiome. In addition to reviewing the clinical findings from FMT studies transferring the fecal microbiota from a healthy donor to an ill recipient, of which there were relatively few studies conducted at the time of the review, the chapter also reviews the findings from preclinical studies investigating the effects of FMT to preclinical animal models from other animal models and from human donors. This systematic review provides context for the power of microbiome manipulation to effect significant changes in psychiatric symptoms both preclinically and clinically, highlighting the improvement observed in all clinical studies, and the transmission of symptoms observed preclinically when FMT was conducted from ill donors to healthy or germ-free recipients (13). The review goes on to once again review potential mechanisms of action and discuss the feasibility, benefits, and drawbacks of large-scale

microbiome manipulation through transplantation as opposed to more subtle methods such as probiotics and prebiotics.

Chapter 4 provides insight into the neurobiological effects of microbial treatments through a systematic review of studies investigating changes in neurophysiology associated with microbial manipulation in both healthy and psychiatric populations (14). With only eight clinical trials in total, five in healthy populations and three in psychiatric populations, this review emphasises the novel nature of this research and the need for further investigations into the neurobiological effects of the gut microbiome. In addition to the low number of trials collecting neuroanatomical or neurophysiological data and studies analysing said data, the neurobiological measures differed significantly between each study, making comparisons difficult. The findings from studies in healthy populations were mixed, with two studies finding no significant changes in their neurobiological measures (15,16), and the remaining three studies finding neurobiological changes relating to the default mode network, working memory performance areas, and brain activity measured through electroencephalography (EEGs) (17–19). As for the studies and analyses derived from clinical trials in psychiatric populations, all found probiotic administration to be associated with significant neurobiological changes in the direction of a healthier profile or correlated psychiatric symptom improvement (20–24). The review goes on to discuss the implications of the specific findings from the studies in relation to psychiatric illnesses and proposed mechanisms of action for gut therapeutics, and the limitations of the research conducted to date.

The deep understanding of the field of microbiome manipulation in psychiatry achieved through the previous literature and systematic reviews informed the primary analysis reported in Chapter 5. The analysis used data from the CANBIND 12: Effects of Probiotics on Symptoms of

Depression study (25) that was started and coordinated by Dr. Caroline Wallace in partnership with Lallemand Health Solutions Inc. before I took over coordination at the beginning of 2020. Dr. Wallace used the primary findings on efficacy and tolerability for her PhD thesis, which allowed me to focus on the analysis of microbial data from stool samples, and neuroimaging data from MRIs collected over the course of the study.

The microbial analysis focused on differences in changes in alpha diversity over the course of the trial between the probiotic and placebo groups, while the neuroimaging analysis focused on group differences in resting state functional connectivity. The alpha diversity analysis displayed mixed results, with phylogenetic diversity and observed operational taxonomic units displaying an increase only in the placebo group, but Shannon's entropy showing an increase in only the probiotic group. These findings are consistent with the literature in the sense that the findings in the literature have been inconsistent and often contradictory (26). A potential explanation for the specific findings from this analysis would be that the probiotic had a stabilising effect on the gut microbiota by resisting the introduction of new bacterial species, while allowing less prolific species already present in the gut to flourish, but the limitations of this study and analysis make it difficult to conclude this with any certainty.

The resting state functional connectivity analysis was based off of the only other study to date investigating changes in resting state functional connectivity associated with probiotic administration in individuals with MDD, which was conducted by Yamanbaeva et al. in 2023 (22). The key difference between the population in that study and this study, is that the participants analysed by Yamanbaeva et al. were taking probiotics in addition to traditional antidepressants, whereas all participants in this analysis were taking solely probiotics. That makes this analysis the first of its kind and presents an opportunity to discern neurophysiological

features associated solely with probiotic administration. An ROI-ROI analysis was conducted between 13 regions of interest, including two clusters identified by Yamanbaeva et al. The regions included in this analysis were all relevant to MDD, including regions implicated in the default mode network and limbic system. This analysis identified 5 functional connections between regions to have significant differences between the probiotic and placebo groups. There was a significant difference between groups in the change in functional connectivity over the course of the trial between the right precuneus and both the left orbitofrontal cortex and right hippocampus, the left superior parietal lobule and the left insula, the right temporal pole and left temporal pole, and the right hippocampus and left amygdala. These observed differences taken together and discussed in the context of differences observed between individuals with MDD and healthy individuals, as well as their respective functions and potential implications of increased or decreased functional connectivity with one another suggests the probiotic intervention used in the CBN12: EPSD study may not be effective in eliciting resting state functional connectivity changes that would traditionally be associated with depressive symptom improvement or response to antidepressant treatment. This is in line with the clinical findings from the study, which did not show any significant differences in the primary outcome measures but could be a consequence of the limitations of this trial such as lack of power due to the small sample size.

6.3 Strengths and Limitations

Within the greater context of the novel field of gut microbiome manipulation in psychiatry, this analysis has some important strengths. One major strength of the CANBIND 12: Effects of Probiotics on Symptoms of Depression study from which this data was collected, was the inclusion of participants that were taking not currently taking any antidepressant or psychotropic medication in addition to the probiotic. The isolated nature of the intervention

allows for the differences observed between the probiotic and placebo groups to be primarily attributable to the probiotic intervention as opposed to potential confounds, such as other medication. Though the limited number of adjuvant studies in the field suggest a combination of probiotic and antidepressant treatments may be more effective clinically than either as monotherapies, this analysis seeks to further understand the mechanisms by which probiotics affect the brain and body, and thus benefits from a sample with no interfering medications.

The mild to moderate illness severity of the participants in this study as opposed to more severe cases of depression is both a strength and limitation. In one way, including participants who are early in their illness trajectory makes these findings more applicable to the expected use of these probiotics outside of a research context. It was expected and observed in the participants interested in the study that patients were more interested in trying probiotics without pharmacological treatment prior to starting antidepressant medications. On the other hand, the neurobiological and physiological characteristics associated with depression may not have been as pronounced in these participants as it would be in individuals further along in their illness trajectory. Since these features may not have been very significant, the effects of the probiotics may have also not been as significant. Exploring these effects in a more severely ill population may yield more pronounced changes associated with the intervention. Another strength of the study was that consumption of probiotic-enriched foods during the course of the study was controlled for. The exclusion criteria specified that participants must not consume probiotic products or fortified foods during the study ensuring that this would not act as a confound during the investigation. That being said, it is possible that there were other dietary changes that occurred in participants which could have influenced their microbiome or other clinical and physiological characteristics. The only way to fully determine the effect of diet on these findings

would be through thorough analysis of the Canadian Diet History Questionnaire which may be done in future analyses.

Despite the many strengths, the study also had some significant limitations. The most significant limitation specific to this study and analysis was the small sample size. This was unavoidable due to issues with recruitment compounded by the COVID-19 pandemic leading to an early termination of the study. fMRI data collection and analysis was further disrupted by the pandemic since the analysis required both baseline and week 8 scans from the participants. This meant that participants who dropped out before week 8 were not included in the analysis, and some participants that completed the study were unable to have a week 8 MRI scan due to the MRI facility being one of the first facilities with restricted access due to the pandemic. Another limitation of the study was the relatively high attrition rate, which could be attributed to the increased burden placed on participants from the high number of assessments. The comprehensive assessments allowed for a full characterisation of participants and a detailed exploration of potential biomarkers but placed a significant burden on the participant by increasing the length of study visits and requiring the participants to undergo assessments that could be unpleasant or burdensome (ie. blood and stool sample collection, polysomnography, and MRIs). Future studies should carefully consider the benefits and drawbacks associated with the number of assessments.

There are also strengths and limitations specific to the analysis discussed in Chapter 5. The fMRI analysis built off of the findings from the only other study investigating changes in resting state functional connectivity associated with probiotic administration in individuals with depression conducted by Yamanbaeva et al. (22). The analysis of the functional connectivity between 13 regions of interest, including the two clusters identified by Yamanbaeva et al.'s seed-

to-voxel analysis, served as an effective way to both, attempt to reproduce the results observed by Yamanbaeva et al. by evaluating the interactions between each ROI and the two clusters, and perform an exploratory analysis by evaluating the interactions between each ROI separately. Despite this, further exploratory analyses may have been informative due to the differences in populations between the two studies. A whole-brain seed-to-voxel based analysis using Yamanbaeva et al.'s original seed of limbic regions may have identified different clusters than the ones identified in their analysis, which could then be associated specifically with probiotic monotherapy. This is a limitation that could be addressed through further analysis of this data, or through future studies.

In addition to the limitations specific to this study, there were also some limitations that are applicable both to the CBN12: EPSD study and studies across the field as seen through the discussions in chapters 1 - 4. One major limitation to the reproducibility of these findings was the probiotic used in the CBN12: EPSD study was trademarked by Lallemand Health Solutions Inc. Though this is common practice in studies partnered with corporations, it means that this study could not be exactly replicated without obtaining the exact same probiotic. Since each study conducted across the field uses specific probiotic products that can be difficult to obtain, the generalisability of their results is reduced, and it is difficult to replicate the findings. This also relates to the lack of consensus on dosages for probiotic studies. The variation between probiotics investigated necessitates dose finding studies for each specific probiotic formulation. Another limitation of the majority of studies in the field is the relatively short length and lack of follow-ups. The duration of the effects of probiotic interventions is not yet fully understood, and a complete understanding would be needed for effective and efficient clinical use.

6.4 Implications and Future Directions

There is a significant body of clinical and preclinical evidence suggesting psychiatric symptoms can be affected by gut microbiome therapeutics. Though this field is novel and still in the early stages, the existing literature suggests that probiotics alone and in combination with psychotropic medication, and fecal microbiota transplantation can be effective treatments for psychiatric illnesses such as MDD. Current literature suggests that probiotics may be more effective when combined with pharmaceutical treatments, such as antidepressants (4), and large-scale modification of the gut microbiome through techniques like fecal microbiota transplantation may be more effective than more subtle therapeutics like probiotics in producing significant, short-term changes in psychiatric symptoms (13). The efficacy of gut therapeutics varies between studies, and though many potential mechanisms of action have been and continue to be proposed, the degree to which each proposed mechanism contributes to the therapeutic effect remains unclear, necessitating further research.

One mechanism of action for gut therapeutics and therapies would be through affecting neuroanatomical or neurophysiological changes. Very few studies have collected neurobiological data from participants receiving gut microbiome therapeutics, particularly in psychiatric populations, but most studies conducted to date observed changes in brain structures, networks, or functioning associated with psychiatric illnesses in the direction of a healthier profile or correlated with symptom improvement (14). The analysis reported in chapter 5 suggests that the probiotic used in the CBN12: EPSD study affected the resting state functional connectivity between various brain regions associated with MDD, but not in the direction of a healthier profile. It would seem that any ameliorative effects of this probiotic, which may be revealed through further trials with larger sample sizes, would not be elicited through effecting change in resting state functional connectivity and may be through other mechanisms of action such as

interactions with the immune and endocrine systems. Further large-scale trials would need to be conducted to determine if these observations are replicated in larger sample sizes and if they could be applied to probiotic monotherapy in general.

The various limitations of research in the field of microbiome manipulation in psychiatry discussed previously have significant implications for future clinical trials. The ideal changes in the field needed to address the limitations would be the development of a probiotic product that could be used by any researcher; tested through large-scale trials in participants without any potentially confounding medications; in various stages of their illness trajectory; with robust clinical, biological, dietary, and neurobiological data collections; and long-term follow-ups. Though trials like this would contribute greatly to advancing the field, they are not practical. A more practical alternative would be studies assessing the differences between probiotics containing bacteria of the same genus but different species or strains to determine the effect of probiotic formulation differences, and thus the generalisability of results between studies using different probiotics. Once it has been determined that studies can be directly compared despite having slightly different probiotic interventions, the concerns surrounding generalisability can be reduced. Following that, studies could share data, or meta-analyses could be conducted, allowing for large scale data to be used to inform guidelines for probiotic research in psychiatry, and determine important information such as effective dose ranges, frequency, and modes of administration. Individual studies would then be free to focus on specific outcome measures of interest, while working within a common framework of understanding and methodology across the field. A multi-site, collaborative study across a research network would also enable the practical collection of large-scale data on the effects of probiotics or other gut microbiome therapeutics on psychiatric symptoms and their effects on biological systems and functions. A

detailed understanding of the effectiveness and primary mechanisms of action of gut microbiome therapeutics could allow for the optimization of their use and the identification of specific populations for which they are most effective, markers of response, or detrimental or enhancing adjuvant treatments.

6.5 Conclusions

With a comprehensive review of the field through various scoping and systematic reviews, and the insights provided through the first analysis of resting-state functional connectivity changes associated with probiotic administration alone in a population with MDD, this thesis provides a detailed overview of the psychiatric application of gut microbiome therapeutics, and their physiological effects. While there is a consensus in the field on the potential to effect psychiatric symptoms through manipulation of the gut microbiome, the evidence is mixed regarding the clinical effectiveness of gut microbiome therapeutics for treating psychiatric illnesses, and the findings from the analysis in chapter 5 suggests they may not effect positive change through altering resting state functional connectivity. The significant limitations common across the research in this field identified and discussed throughout the thesis, and suggested future directions, can inform the development of future research questions and investigations. Gut microbiome manipulation has the potential to greatly impact individuals suffering from psychiatric illnesses by providing an alternative treatment and augmenting existing treatments, and an in-depth understanding of the physiological effects of this manipulation may inform our understanding of interplay between psychiatric illnesses and the body.

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