DR. JESSICA EMILY GREEN (Orcid ID : 0000-0002-1219-8910)

DR. ANDREW A NIERENBERG (Orcid ID : 0000-0003-2897-0458)

Article type : Editorial

FMT for psychiatric disorders: following the brown brick road into the future

Jessica E. Green[1, 2, 3], Michael Berk[1,4-7], Amy Loughman[1], Wolfgang Marx[1], David Castle[4,8], Amelia J. McGuinness[1], John F. Cryan[9], Andrew A. Nierenberg[10-11], Eugene Athan[1,7,9], Christopher Hair[7], Felice Jacka[1,13-15]

Corresponding Author: Jessica Green,
jegreen@deakin.edu.au
Deakin University
School of Medicine
PO Box 281
Geelong Victoria 3220

1. Deakin University, IMPACT, the Institute for Mental and Physical Health and Clinical Translation, Food & Mood Centre, School of Medicine, Barwon Health, Geelong, Australia
2. Monash Alfred Psychiatry Research Centre (MAPrc), Central Clinical School, Faculty of Medicine Nursing and Health Sciences, Monash University
3. Department of Psychiatry, Peninsula Health, Frankston, Australia
4. Department of Psychiatry, University of Melbourne, Parkville, Australia
5. Orygen Youth Health Research Centre and the Centre of Youth Mental Health, Melbourne, Australia
6. The Florey Institute for Neuroscience and Mental Health, Parkville, Australia
7. Barwon Health, Geelong, Australia

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/BDI.13124

8. Scientific Director, Centre for Complex Interventions, Centre for Addictions and Mental Health; and Professor, Department of Psychiatry, University of Toronto, Toronto, Canada
9. Department of Anatomy and Neuroscience, University College Cork and APC Microbiome, Ireland
10. Dauten Family Center for Bipolar Treatment Innovation, Department of Psychiatry, Massachusetts General Hospital, Boston, MA, USA
11. Harvard Medical School, Boston, MA, USA
12. School of Medicine, Deakin University, Geelong, Australia
13. Centre for Adolescent Health, Murdoch Children's Research Institute, Royal Children's Hospital, Parkville, Australia
14. Black Dog Institute, Melbourne, Australia
15. James Cook University, Townsville, Australia

The field of microbiota-gut-brain axis research and medicine, including the potential and application of faecal microbiome transplants (FMT), continues to accelerate (1). Here we update the evidence regarding the use of FMT in psychiatry, discuss the many clinical and methodological challenges, and make recommendations to guide further high-quality research.

An update on microbiota-gut-brain axis research

Microbiota-gut-brain research aims to clarify the complex and interacting mechanisms connecting the gut, and its resident microbiota to the central nervous system (CNS). Multiple interacting pathways and mechanisms are implicated. These include the immune system, oxidative stress, brain plasticity (hippocampal neurogenesis), the tryptophan-kynurenine pathway, the hypothalamic-pituitary-adrenal axis, and neurotransmitters, among others. These pathways overlap with those implicated in the neurobiology of mood and related disorders. Furthermore, an increasing number of studies describe specific changes in gut microbiota in diverse neuropsychiatric conditions. These include Parkinson's disease, Alzheimer's dementia, multiple sclerosis, epilepsy, Tourette Syndrome, chronic fatigue syndrome/myalgic encephalomyelitis, Guillain-Barre syndrome, stroke, Huntington's Disease, autism spectrum disorder, bipolar disorder, major depressive disorder, anxiety and neuropathic pain. Due to their emerging role in the pathogenesis of neurological and psychiatric diseases, the gut microbiota represents a novel therapeutic treatment target.

A recent meta-analysis of 24 clinical trials identified a small yet significant improvement in depressive symptoms for probiotics compared to placebo. However, this effect increased to medium-to-large when restricted only to studies of clinical samples of MDD. This provides further support for an effect of gut microbiome modulation on mental health outcomes for people with clinical depression. New efforts are also

being directed toward developing biotherapeutics targeting the intestinal microbiota with the goal of treating depression and anxiety. As previously noted (1), suggestive of causal relationships, FMT from depressed humans administered to microbiota-depleted mice induces depression-like behaviours in the mice (1). FMT from humans with schizophrenia into rodents also induces a schizophrenia phenotype, including biochemical signatures posited to be associated with schizophrenia (1). In this context, we review the emerging data from human studies of FMT and consider limitations, challenges and opportunities.

With respect to bipolar disorder, there are compelling data demonstrating a link between the disorder and gut microbiota. To begin, bipolar disorder is highly comorbid with gut symptoms. A meta-analysis of 177,117 individuals with IBS and 192,092 healthy controls revealed a significantly higher rate of bipolar disorder in those with IBS compared with healthy controls (OR = 2.48, $p < 0.001$). In addition, differences in gut microbiota in individuals with bipolar disorder have been observed compared with healthy controls, for example, decreased relative abundance of *Faecalibacterium*. There is also evidence suggesting the gut microbiome may impact response to medications in bipolar disorder. Conversely, medications for bipolar disorder such as lithium, anticonvulsants and antipsychotics have been shown to alter gut microbiota. Finally, preliminary studies have suggested that probiotics may improve cognitive function and reactivity in euthymic BD, and reduce hospitalisation rates following manic episodes.

Update on FMT research

FMT is a technique by which gut microbiota are transferred from the intestine of a healthy donor into another individual with the goal of restoring healthy intestinal flora. The treatment is gaining increasing attention across several indications believed to be linked with disturbed intestinal microbiota. Given the compelling field of microbiota-gut-brain research described above, FMT holds promise in the treatment of psychiatric disorders through its potential to modify the gut microbiota.

FMT is an established, safe and effective means of treating recurrent or treatment refractory *Clostridioides difficile* infection, with cure rates of up to 90%. But is FMT is safe and efficacious in disorders other than *Clostridioides difficile* infection? Our recent systematic review and meta-analysis (2) reviewed all controlled studies of FMT for health conditions other than *Clostridioides difficile* infection. Twenty-six controlled studies were included, spanning a large range of clinical indications including inflammatory bowel disease, functional gut disorders, metabolic disorders and obesity, hepatic disorders and antibiotic-resistant organism infections. There were sufficient data to conduct meta-analyses in acute ulcerative colitis (UC), and irritable bowel syndrome (IBS): these confirmed that FMT is efficacious in treating active UC, significantly improving rates of clinical and endoscopic remission and clinical response. However, the data pertaining to IBS, which is highly comorbid with common mental disorders, was equivocal, with three studies favouring FMT and two favouring the control condition. Finally, we reported preliminary evidence supporting the efficacy of FMT in treating hepatic disorders, metabolic syndrome, and antibiotic-resistant organisms (2). Since our review was published, further promising data have emerged, including a pilot study evaluating the ability of FMT to modulate treatment response in immunotherapy-refractory melanoma patients, with associated favourable changes in the immune system which have implications for cancer treatment (3).

Regarding safety, our review found that serious adverse events were – unexpectedly - more often reported in control group participants (n = 43) compared with FMT group participants (n = 26). This may be because FMT was effective at treating the underlying disorder in many cases and, thus, the greater number of serious adverse events observed in the control groups reflected the underlying and untreated disease processes themselves. Rates of mild to moderate adverse events were similar across both groups (2).

Our systematic review and meta-analysis (2) also revealed a critical literature gap, in that we did not identify any controlled trials in humans evaluating FMT for psychiatric disorders. Three of our included studies conducted in IBS populations also evaluated mental health outcomes, one reported a significant difference in favour of the FMT group ($p<0.05$) and two showed trends toward a benefit, but the results were not statistically significant.

Meyyappan et al. recently published a systematic review of preclinical and clinical psychiatric outcome measures in FMT studies (4). This review identified eight clinical studies, nine preclinical studies with human donors, and 11 exclusively preclinical studies. Of the eight clinical studies, four were open label, three were case studies, and only one was a randomised controlled trial (RCT). This RCT was in IBS patients who had not been assessed for depression/mental illness; quality of life was used as a proxy psychiatric outcome measure. Furthermore, of the eight clinical studies, only three case studies assessed or included patients with a confirmed diagnosis of a mental illness (major depressive disorder in two cases, and anorexia nervosa in one case). All five of the remaining clinical studies were in IBS populations (psychiatric comorbidity was not assessed). All included studies (clinical and preclinical) found a decrease in depressive and anxiety-like symptoms and behaviours associated with transplantation of healthy microbiota. However, none of the studies were specifically designed to measure psychiatric outcomes, were suitably powered to measure psychiatric outcomes, or reported statistically significant changes in psychiatric outcome measures. Thus, this evidence - whilst promising - is very preliminary.

It is also important to note that due to the specific search criteria used, some relevant publications were not included in the abovementioned systematic reviews. In addition to the autism spectrum open label trial discussed in our 2020 review (1), there is a case series of FMT in three cases of comorbid depression and IBS. Following FMT, depressive symptoms subjectively improved in all three cases; however, rating scales were not used. In addition, a recently published Australian case study reported that enema-delivered FMT effectively treated long-standing Bipolar I Disorder. These additional preliminary studies support further research for FMT in psychiatric disorders.

There are also interesting pilot data evaluating the role of FMT in early life, where infants delivered by caesarean section have potentially adverse differences in gut microbiota profiles to those delivered vaginally. These differences have been associated with several adverse outcomes in caesarean-delivered infants, such as obesity, attention deficit hyperactivity disorder and autism spectrum disorder. 'Vaginal seeding', involves exposing caesarean-born infants to their mother's vaginal microbiome post birth. This technique is controversial, has safety concerns, and is not effective in correcting the difference between faecal microbiomes between caesarean-born and vaginally-delivered infants. In contrast, in a recent pilot study of

seven infants, FMT from their mother corrected the differences in gut microbiota between Caesarean-delivered and vaginally-delivered infants, as well as appearing to be safe and well tolerated (5). These findings may have implications for disease prevention. However, it must also be noted that these data are preliminary and need to be considered with caution before any consideration of adoption into mainstream practice. We note that this approach and interventions that target the maternal microbiota, with the aim of improving infants' microbiota and related health outcomes, are currently the subject of active research attention in our centre.

Challenges and prospects in FMT research:

Notwithstanding the need for experimental human data evaluating FMT for psychiatric disorders, there are significant barriers to conducting FMT research in general. Because this field is so new and data are lacking, there is no consensus around many aspects of methodology including: top down or bottom up routes of administration (e.g., encapsulated/oral, endoscopic, colonoscopic, enema); use of adjunctive treatments (e.g., antibiotics, change in diet/fasting prior to intervention, use of bowel preparation); discrepancies between donor screening protocols used, FMT manufacture procedure and storage requirements (e.g., aerobic vs anaerobic preparation, frozen vs fresh vs lyophilised, use of cryoprotectant, concentration of bacteria/microbiota in the product); single donor vs pooled donation; and FMT "dosage". Any one of these factors has the potential to have an impact on the efficacy of the product.

FMT is a highly effective treatment for *Clostridioides difficile* infection, such that the methodological factors listed above are of little relevance in its treatment. A wide spectrum of approaches appear effective. However, closer attention to FMT methodology may be required in the case of chronic disorders, which we hypothesise may be linked with chronically disrupted pathological intestinal microbiota profiles. To use a botanical analogy, the intestinal microbial state associated with *Clostridioides difficile* infection could be thought of as a forest that has been decimated by bushfires. In this type of environment, it would not be hard to germinate new species, as there are no plants to compete with the new crop. As such, the cultivation technique utilised could vary widely, all with successful outcomes. However, the story is different in the case of trying to alter an existing complex ecosystem, such as the disrupted microbiota profile which may be associated with chronic disease states such as major depressive disorder. This could be likened to trying to restore a natural rainforest ecosystem, after it has been destroyed by waste pollution and the introduction of invasive pest species. Specific cultivation and restoration techniques would be pertinent here. Likewise, we suggest that methodology will be more critical in the use of FMT for mental disorders, in a way that it may not be in *Clostridioides difficile* infection.

Whilst few clinical data are available to guide us, preservation of anaerobes in the FMT manufacturing process may, theoretically, be of importance. They include most of the bacteria in the human gastrointestinal tract and may therefore comprise a key part of the 'active ingredient' of effective FMT. Thus, a manufacturing technique that preserves anaerobic bacteria to as great an extent as possible may be important in optimising the efficacy of FMT. As such, many labs are striving to utilise anaerobic techniques in FMT manufacture. Yet this is also not straightforward, as preservation of anaerobes poses technical challenges for the manufacture process and

there continues to be a lack of clarity around how to best achieve or measure this. A truly complete 'end to end' anaerobic procedure has yet to be developed, as from the moment the stool is passed it is exposed to oxygen, which acts immediately on exposed faecal anaerobes. While some measures can be taken to improve the amount of viable anaerobic bacteria, such as use of an anaerobic container into which the faeces can be passed and stored prior to processing, and processing faeces under an anaerobic hood, these processes are both expensive and laborious..

Moreover, both the route of administration (i.e. 'top down' versus 'bottom-up' approaches) and dosage may also influence how effectively the donor FMT 'engrafts' to the recipient's intestine and for how long this engraftment persists. There are still too few data on which to base decisions and inform optimal trial designs. Functional and psychiatric disorders are also likely to have smaller effect sizes than *Clostridioides difficile* infection and there are high rates of placebo response in psychiatric research, especially with invasive procedures/interventions. As such, large sample sizes and randomised, well-blinded designs are required in clinical trials. Given the many unanswered questions regarding optimal FMT methodology, more studies – potentially harmonising aspects of study protocols to afford increased sample sizes and comparison of dosing and delivery strategies – are needed before consensus will be reached.

Another barrier to the clinical application of FMT is the recent change in the regulations around FMT manufacture imposed by The Australian Therapeutic Goods Administration (TGA). TGA approval requires several stringent criteria to be met with high associated costs. For example, it is necessary for laboratories to obtain Good Manufacturing Practice (GMP) approval through a rigorous accreditation process conducted by the TGA. . Regulation of FMT is welcomed as it ensures a high standard of product, appropriate safety standards, governance, and oversight. Larger and adequately resourced stool banks can use economy of scale to set up processes that meet the TGA requirements, ensuring a high-quality, consistent product. The Adelaide-based stool bank, BiomeBank, has been the main supplier of FMT to South Australia for seven years and interstate for two years, and the Australian Blood Service (Lifeblood) is also in the process of setting up a national stool bank. These services will facilitate widespread application of FMT in clinical practice, should efficacy studies confirm its utility in conditions other than *Clostridioides difficile*. In the USA, the regulatory landscape is different, with the US Food and Drug Administration (FDA) not acting as an official regulator of FMT, and not providing official guidelines regarding FMT manufacture and donor screening. Thus, in the USA, regulatory approval is the responsibility of each health network or practitioner.

Safety is another consideration in FMT research. FMT is considered a generally safe treatment with guideline-concordant manufacture and has been widely used in routine clinical practice for thousands of individuals over many years, in many populations and for many indications, with no major safety concerns (2). Furthermore, FMT seems safe in healthy recipients, not merely those with *Clostridioides difficile* infection and other health disorders. This finding is important as, when a participant has physical symptoms relating to an underlying condition, it may be difficult to identify which adverse event is related to the FMT and which is related to the condition itself. This is not a new issue in psychiatry, with considerable research showing that most adverse events reported with antidepressant use, for example, are not related to the medication itself. Thus, the

establishment of safety in healthy populations removes these potentially biasing factors and affords confidence in the application of FMT.

However, while FMT has been employed and formally studied for a decade, there is a lack of long-term safety data on FMT beyond this timepoint. Additionally, the US Food and Drug Administration has issued warnings**.** On 13th June 2019 the FDA reported two cases of immunocompromised patients in whom antibiotic-resistant organisms (specifically, Extended Spectrum Beta Lactamase-producing *Escherichia coli* (ESBL)) were transferred via FMT, resulting in one death. In these immunocompromised cases, current accepted standards for donor screening were not adhered to in that donor faeces were not screened for antibiotic-resistant organisms. Another warning was issued on 12th March 2020, reporting another six cases of transmission of antibiotic-resistant organisms via FMT supplied by a US-based stool bank (two cases of enteropathogenic Escherichia coli and four cases of Shiga toxin-producing Escherichia) and two deaths in recipients of FMT, but in which FMT was not confirmed to be the cause of death. These serious incidents reinforce the importance utilising rigorous screening protocols and the value of regulation of FMT practice such as the TGA providing regulatory oversight, and the utility and safety of stool banks that adhere to these stringent guidelines. It is also worth noting that parasites and viruses including COVID-19 can be transmitted via stool, necessitating additional and regularly updated screening measures for those manufacturing FMT.

Finally, funding is a perennial challenge for clinical research. Is difficult to obtain funding for novel and cutting-edge research, as by its very nature, it lacks precedent and robust existing data. FMT is also expensive to produce as it requires stringent screening and safety protocols for donors in addition to Good Manufacturing Practice accredited manufacturing costs. Microbiome testing is also specialised and expensive.

Current and imminent clinical research on FMT in psychiatry

We now turn our attention to current activities in the field of FMT research in psychiatry. Projects mentioned in our 2019 article (1) have now progressed. Professor Valerie Taylor in Calgary, Canada, is nearing completion of an RCT evaluating the efficacy of colonoscopic FMT compared to placebo for the treatment of bipolar depression. Her group also recently commenced recruitment for a RCT of encapsulated FMT for unipolar depression. In addition, we have identified several newly registered FMT trials in the field of neuropsychiatry, including trials in Sjogren's Disease, anorexia nervosa, Chronic Fatigue Syndrome, depression in schizophrenia, and depression and anxiety symptoms in individuals with constipation. In addition, planning is underway to run a pilot study of FMT for depression in Geelong this year (ANZCTR registration number: 12621000932864). This study will lay the groundwork for a fully-powered RCT to commence in 2022 ("the Moving Moods Study"), designed to evaluate the efficacy and cost-effectiveness of FMT for major depressive disorder in adults.

Recommendations

While there is much work to be done to establish a consensus around optimal methodologies, including mode of delivery, choice of placebo, dosage, FMT manufacture details, and use of adjuvant treatments, the emerging data for FMT are positive and support further clinical research in psychiatry. We urge collaboration with existing groups developing expertise in this field to harmonise protocols as much as possible, using comparable rating scales/tools, to afford the capacity to pool data and increase statistical power. Adherence to high quality RCT study design is essential, including attention to an appropriate control group and adequate sample sizes.

While the cost of FMT products and delivery via colonoscopy is currently high, economies of scale possible through existing and emerging stool banks, will reduce these costs. The new regulatory frameworks to ensure safety, consistency and quality of product also affords confidence in the safety of FMT. With several quality trials currently underway or planned for the near future, we eagerly anticipate stepping further down the brown brick road into a promising future.

Author Contributions:

JG prepared the manuscript and completed edits in response to reviewer and co-author feedback. FJ reviewed and edited the manuscript and acted as senior author. All authors reviewed, edited and approved the final manuscript.

Acknowledgements:

JG is supported by an IMPACT PhD Scholarship through IMPACT, Deakin University.

FNJ is supported by an NHMRC Investigator Grant (#1194982). She has received: (1) competitive Grant/Research support from the Brain and Behaviour Research Institute, the National Health and Medical Research Council (NHMRC), Australian Rotary Health, the Geelong Medical Research Foundation, the Ian Potter Foundation, The University of Melbourne; (2) industry support for research from Meat and Livestock Australia, Woolworths Limited, the A2 Milk Company, Be Fit Foods; (3) philanthropic support from the Fernwood Foundation, Wilson Foundation, the JTM Foundation, the Serp Hills Foundation, the Roberts Family Foundation, the Waterloo Foundation and; (4) travel support and/or speakers honoraria from Sanofi-Synthelabo, Janssen Cilag, Servier, Pfizer, Network Nutrition, Angelini Farmaceutica, Eli Lilly, Metagenics, and The Beauty Chef. Felice Jacka has written two books for commercial publication.

MB is supported by a NHMRC Senior Principal Research Fellowship (1156072). MB has received Grant/Research Support from the NIH, Cooperative Research Centre, Simons Autism Foundation, Cancer Council of Victoria, Stanley Medical Research Foundation, Medical Benefits Fund, National Health and Medical Research Council, Medical Research Futures Fund, Beyond Blue, Rotary Health, A2 milk company, Meat and Livestock Board, Woolworths, Avant and the Harry Windsor Foundation, has been a speaker for Abbot, Astra Zeneca, Janssen and Janssen, Lundbeck and Merck and served as a consultant to Allergan, Astra Zeneca, Bioadvantex, Bionomics, Collaborative Medicinal Development, Janssen and Janssen, Lundbeck Merck, Pfizer and Servier – all unrelated to this work.

WM is currently funded by an Alfred Deakin Postdoctoral Research Fellowship and a Multiple Sclerosis Research Australia early-career fellowship. Wolfgang has previously received funding from the NHMRC, Clifford Craig Foundation, Cancer Council Queensland and university grants/fellowships from La Trobe University, Deakin University, University of Queensland, and Bond University, received industry funding and has attended events funded by Cobram Estate Pty. Ltd, received travel funding from Nutrition Society of Australia, received consultancy funding from Nutrition Research Australia, and has received speakers honoraria from The Cancer Council Queensland and the Princess Alexandra Research Foundation.

AL has received grants, fellowships and research support from the University of New South Wales, the University of Melbourne, RMIT University, Deakin University, the National Health and Medical Research Council (NHMRC), Australian Academy of Science, National Institutes of Health (NIH), and The Jack Brockhoff Foundation. AL has received honoraria and travel funds from Sydney University, the University of Technology Sydney, American Epilepsy Society, Epilepsy Society of Australia, International Human Microbiome Congress, European Society of Neurogastroenterology, Australian and New Zealand College of Anaesthetists, Falk Foundation and Fonds de la Recherche Scientifique (FNRS).
AJM has received funding from Australian Rotary Health via the Australian Rotary Health/Ten Island Tassie Tag Along Tour Funding Partner PhD Scholarship.

References

- 1. Green J, Castle D, Berk M, Hair C, Loughman A, Cryan J, et al. Faecal microbiota transplants for depression - Who gives a crapsule? The Australian and New Zealand journal of psychiatry. 2019;53(8):732-4.
- 2. Green JE, Davis JA, Berk M, Hair C, Loughman A, Castle D, et al. Efficacy and safety of fecal microbiota transplantation for the treatment of diseases other than Clostridium difficile infection: a systematic review and meta-analysis. Gut microbes. 2020;12(1):1-25.
- 3. Baruch EN, Youngster I, Ben-Betzalel G, Ortenberg R, Lahat A, Katz L, et al. Fecal microbiota transplant promotes response in immunotherapy-refractory melanoma patients. Science. 2021;371(6529):602.
- 4. Chinna Meyyappan A, Forth E, Wallace CJK, Milev R. Effect of fecal microbiota transplant on symptoms of psychiatric disorders: a systematic review. BMC Psychiatry. 2020;20(1):299.
- 5. Korpela K, Helve O, Kolho KL, Saisto T, Skogberg K, Dikareva E, et al. Maternal Fecal Microbiota Transplantation in Cesarean-Born Infants Rapidly Restores Normal Gut Microbial Development: A Proof-of-Concept Study. Cell. 2020;183(2):324-34.e5.