Transfer of altered behaviour and irritable bowel syndrome with diarrhea (IBS-D) through fecal microbiota transplant in mouse model indicates need for stricter donor screening criteria

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Experimental studies performing fecal microbiota transplants (FMTs) from human to animals and between animals is providing enlightening information with regards to phenotypical characteristics that can potentially be influenced by the microbiome. A recent study by De Palma (1) has shown that fecal material from humans with irritable bowel syndrome (IBS) with diarrhea (IBS-D) invoked physical changes in the intestinal environment and in behavior. This is an interesting outcome, given the questions that have been raised about the relevance of certain models' in light of the differences between the rodent gut physiology, the microbiome and the human condition (2,3).

IBS is characterized by discomfort of the abdomen associated with altered bowel functions, where structural and biochemical abnormalities are absent (4). IBS is prevalent in 11.2% of the global population with more women being affected than men (5). Patients with IBS have significantly higher rates of anxiety and depression than healthy people (6) and they have been shown to have a reduced quality of life (7). IBS can be broken down further into different subtypes including: IBS-D (the focus in this De Palma study), constipation-predominant IBS (IBS-C), mixed-IBS (IBS-M) and unsubtyped IBS (IBS-U).

Since IBS is a condition of the gastrointestinal tract, it has been postulated that the disease is caused by alterations in the composition of the gut microbiome. Multiple studies have been conducted trying to determine if patients with IBS have a microbiota that differs from people without the disease. The most conserved finding has been a decrease in diversity of the gut microbiota (8-12). Attempts to identify specific differences in the relative abundances of the gut microbiota of IBS patients have found various results for each study. There is also the possibility that changes in the gut microbiota in IBS patients may be a result of the syndrome, rather than a cause of it, as a variety of other factors may predispose someone to develop IBS, such as genetics, food sensitivities, and food poisoning. Due to the evidence that the microbiota plays a role in the development of IBS, De Palma *et al*. hypothesized that germ-free mice given a FMT from an IBS-D patient would have altered behaviour, gastrointestinal transit, gut barrier function, and immune activation.

In the De Palma study, stool was collected from five healthy controls (HCs) (two men and three women, mean age: 42 years) and eight IBS-D patients (three men and five women, mean age: 40 years). Patients with IBS were diagnosed using the Rome-III criteria and Bristol stool form scale (≥6), with more than three bowel movements per day and symptoms for at least 2 years. Of the IBS-D patients, four were characterized as having anxiety by the Hospital Anxiety and Depression Scale (HADS) (HADS score, 11–14) and four IBS-D patients did not have anxiety (HADS

score, 5–7). Eight- to 10-week-old germ-free National Institutes of Health (NIH) Swiss mice were gavaged with stool from either HCs or IBS-D patients with or without anxiety (at least ten mice per donor).

Three weeks after the FMT, the mice were assessed for changes in their behaviour using the step-down and light preference tests. Changes in gastrointestinal transit time were measured by gavaging five small metal beads with barium solution and imaging using a videofluoroscope approximately 3 hours later. A scoring system based on the location of the beads in the gastrointestinal tract was used on the resulting images to determine the gastrointestinal transit score.

The mice were then sacrificed and a section of the colon from each mouse was analyzed for barrier function using the Ussing chamber technique. Briefly, a radioactive probe (^{51}CR-EDTA) was added to the luminal side of the device and the transport of the probe across to the serosal side was measured after 2 hours to determine the proportion of transport that took place. An array of proinflammatory cytokines and mRNA expression of β-defensin 3 and CXCR3 were measured from colonic tissue sections from each mouse. In addition to clinical outcomes, De Palma *et al.* conducted 16S rRNA gene sequencing analysis on fecal and cecal samples from the colonized mice (3 weeks post-transplant) and human donors.

The authors found that mice colonized with microbiota originating from IBS-D patients with anxiety spent longer amounts of time in the dark compartment during the light-preference test than mice colonized by HCs and IBS-D patients without anxiety, as well as longer latency in stepping off an elevated platform in the step-down test, indicating these mice had an altered behaviour when colonized with the microbiota of IBS-D patients with anxiety. Faster transit time was observed in mice colonized by the microbiota of IBS-D patients than HCs. Gut barrier function was impaired in mice colonized by IBS-D patients' microbiota and the authors found that there was increased ion transport in IBS-D colonized mouse colonic tissue (but not jejunal tissue) compared to HCs.

The cytokine profile of mice colonized with the microbiota of IBS-D patients did not differ from those colonized by HCs. An increase in CD3+ T lymphocytes was observed in the IBS-D with anxiety mice compared to IBS-D without anxiety and HC mice. A total of 22 genes involved in inflammatory pathways were found to be upregulated in IBS-D mice. 16S rRNA gene sequencing analysis showed that mice colonized by the same donor had a composition of microbiota that was similar, but it could not be concluded with certainty if mice colonized by IBS-D or HC patients had differing microbial compositions in their guts. The authors suggest that due to the higher relative abundances of DNA sequences of certain organisms being present in the IBS-D patients with and without anxiety and HC, there may be indicator species for IBS-D and anxiety. They found that humans with IBS-D had a higher relative abundance of the genus *Blautia* and humans with anxiety had higher relative abundances of the genera *Blautia*, *Coprococcus*, *Streptococcus*, and the species *Clostridium butyricum* and *Eggerthella lenta*. Mice colonized by IBS patients had higher relative abundances of the genera *Oscillospira*, *Bacteroides*, and the species *Clostridium citronia* and mice with anxiety had higher relative abundances of the genus *Oscillospira* and the species *Bacteroides fragilis*, *Akkermansia muciniphila*, *Shewanella algae*, and *Blautia producta*.

The De Palma study provides promising data with regards to advancing our understanding of the microbial contribution to the pathophysiology of IBS-D and its psychiatric comorbidities. Past research from these authors demonstrated antibiotics administered to mice altered their behaviour (13) and this was the basis for their hypothesis that an altered microbiota could contribute to the psychiatric conditions commonly observed in IBS patients. Overall, they found that the phenotypes of altered behaviour, decreased gastrointestinal transit time and immune activation could be conferred to mice through an FMT from IBS-D patients.

A few limitations of the data presentation are worth noting. Large amounts of variability were observed within mouse groups for intestinal transit, step-down and light preference test scores. Therefore, presenting the individual data points would have allowed enhanced visualization of the data distribution, as some mice were potentially responders to the treatment and others were not. In the supplement, data for the step-down and light preference tests of germ-free mice were included to show that mice colonized with the microbiome from patients with IBS-D with anxiety had altered behaviour. Gastrointestinal transit scores and immune activation in the germ-free mice were not included within the paper or the supplement and baseline values for these mice could not be determined. It would be interesting to know whether the mice colonized by IBS-D patients truly have increased gastrointestinal transit scores and immune activation or did the mice colonized by HCs have reduced values compared to a germ-free mouse making the IBS-D colonized mice appear elevated?

Mice receiving an FMT from the same donor had microbiota compositions that clustered together 3 weeks after FMT. Mice colonized by IBS-D patients (with and without anxiety) and HCs did not cluster separately during 16S rRNA gene sequencing analysis, indicating in this analysis that there is not a discrete microbiota pattern associated with IBS-D colonized mice. This finding could be due to the small sample size of this study: with only eight IBS-D donors. This study did not find that the microbiota of HCs and IBS-D patients clustered separately during analysis, but an attempt was made to identify organisms that may be linked to IBS-D. Again, given the numbers in both arms of the study (five HCs *vs.* eight IBS-D patients), it cannot be said with certainty if these organisms are associated with the disease state, but the physiological changes observed in the mice indicate that human fecal contents can confer IBS-D-like symptoms and behavioural changes. The authors plan to use larger sample sizes of human participants in the future, which have the potential to realize exciting results.

This study raises questions of other interesting animal studies that could be performed. It would be interesting to undertake repeated FMT passaging from the anxiety-afflicted mice into other germ-free mice to determine the minimal microbial contribution to the induced psychological state, which may give clues to specific microbial contributors. It could be worthwhile to determine if the physiological changes observed in the IBS-D colonized mice could be reversed with antibiotics or a second FMT from a HC. Double-blinded randomized control trials treating IBS-D are in progress and results from these studies will be more definitive in determining if IBS-D can be treated with FMT. The authors acknowledged that human host factors could have been the source of the physiological changes observed in the germ-free mice, and in future experiments this problem could be solved by cultivating the microbiota from a human stool in a chemostat system and gavaging the mice with this material.

What could the results of this study mean for the clinical use of FMT? For its use in treating recurrent *Clostridium difficile*, the screening process for donors varies between clinics, and acceptance rates can range from 10% to 37% (14,15). Screening for IBS-D and any gastrointestinal disease is common practice for all FMT clinics, however, there are discrepancies in screening protocols when it comes to psychiatric history. Stool banks that distribute a large number of samples to many different clinics are of a particular concern because they don't necessarily screen for psychiatric conditions. Use of validated scoring systems to quantify anxiety will have to be utilized. However, it is unclear if even mild degrees of subclinical variation in psychiatric morbidity may be transferred and so identification of the optimal screening systems will require a comprehensive review and ongoing monitoring of patient cohorts for psychiatric morbidity post-transplant. In particular, it is unknown whether some FMT donors may carry a fecal microbiome capable of inducing psychiatric or functional gastrointestinal morbidity in a susceptible host, but due to host factors in the donor the phenotype is not expressed and so would not be identified by symptomatic screening. The ability to develop microbiome directed screening rather than donor phenotype screening will require further study.

It is uncertain whether the gut and psychological changes seen in this animal model are relevant to the human FMT model. Human FMTs do not occur in a baseline germ-free environment and although the recipient microbiome approaches that of the donor post-transplant, many differences persist (16) and thus whether the phenotypes described here would be transferred between humans is unclear. Furthermore, it is unknown if the stage of development of the FMT recipient impacts susceptibility to the microbial changes. The impact of gut microbiome changes on symptoms when performed in childhood *vs.* adulthood would require investigation.

This study showed that significant psychological and gut functional changes could be transferred to mice from diseased humans via FMT. While it may be possible that animal models are more susceptible to phenotype alterations induced by microbes in an FMT, or that humans are not typically given an FMT in a germ-free state or at a pivotal developmental stage of their lives, it may still be wise at this relatively early stage of FMT clinical use to assure that the exclusion criteria for donors for FMT include IBS and history of mental illness.

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None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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