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SPECIAL REPORT

Microbiota transplant therapy and autism: lessons for the clinic

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ABSTRACT

Introduction: The purpose of this review is to discuss Microbiota Transplant Therapy (MTT), a type of intensive intestinal microbiota transplantation (IMT), for people with autism spectrum disorders (ASD) and chronic gastrointestinal disorders (constipation and/or diarrhea).

Areas covered: This paper briefly reviews IMT, gastrointestinal symptoms and gastrointestinal bacteria in children with ASD, and results and lessons learned from intensive MTT for autism.

Expert opinion: An open-label study and a two-year follow-up suggest that MTT is relatively safe and effective in significantly reducing gastrointestinal disorders and autism symptoms, changing the gut microbiome structure, and increasing gut microbial diversity. Further research with larger, randomized, double-blind, placebo-controlled studies is warranted.

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1. Introduction

Approximately 30-50% of the children and adults with autism spectrum disorders (ASD) have chronic gastrointestinal symptoms, typically constipation, diarrhea, and alternating constipation-diarrhea [1–4], with some studies reporting up to 78% when including mild severity [4]. These conditions can persist for many years or even decades, resulting in substantial pain and discomfort and a long-term decrease in quality of life. Most of these symptoms are considered functional disorders, i.e., disorders of gut-brain communication such as irritable bowel syndrome, and several mechanisms linking functional disorders and gut microbiota have been postulated [5]. Some studies have reported that children with ASD who also have gastrointestinal (GI) disorders have more severe autism or autism-related symptoms than do children with ASD who do not have GI disorders [2,6,7], and it is suspected that some of these gastrointestinal disorders may be due to dysfunctional activity of the gut microbiota. If the GI symptoms can be treated, improvement in ASD-related symptoms and behavior could be expected.

One promising treatment for GI problems in children with ASD is intestinal microbiota transplantation (IMT), also commonly known as fecal microbiota transplantation (FMT), in which microbiota from feces of healthy donors is transplanted into patients to attempt to restore a healthy microbiota composition and function to treat their gastrointestinal disorder [8–11]. In current clinical practice, the main indication for IMT is recurrent *Clostridium difficile* infection (rCDI), a condition that is associated with repeated treatments with antibiotics that result in near-obliteration of the patient's indigenous intestinal microbiota. In most cases, a single administration of healthy donor intestinal microbiota results in normalization of the intestinal microbial community structure in the rCDI recipients and restoration of colonization resistance to *C. difficile* [8–11]. Given the potential importance of intestinal microbiota in pathogenesis of a multitude of diseases and the potential of IMT to induce a major change in the composition and functionality of intestinal microbiota, there has been growing interest in applying IMT to indications other than *C. difficile*. In considering such application, it is critical to distinguish IMT regimens that aim to restore a decimated microbiota, as seen in rCDI patients, versus attempts to displace an established microbiota [12]. In general, much more intensive regimens involving multiple administrations of IMT are required to achieve the latter, as illustrated by clinical trials in using IMT for treatment of inflammatory bowel disease [13].

There is increasing interest in the potential of microbiota-based therapeutics toward disorders associated with altered gut-brain communication. A number of neurologic and neurodevelopmental disorders are associated with altered composition of microbiota (dysbiosis) and weakened gut barrier function [14]. In addition, some of the microbial products, e.g., various metabolites of aromatic amino acids, have the potential to be neuroactive and affect the functions of the enteric and central nervous systems [15–19]. Therefore, it is conceivable that by radically changing a dysbiotic microbiota to a healthier composition, IMT may impact such disorders.

Interestingly, two large studies [20,21] involved treating chronic fatigue patients with a subset of bacteria grown from human stool. Those studies observed response rates of 41% (for two consecutive days of therapy) and 70% (two consecutive days, and two

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additional days if needed) for chronic fatigue symptoms, and even higher response rates for GI symptoms, with benefits reported lasting up to 12–15 years in some cases. Similarly, a case-report [22] of six rounds of IMT resulted in 'a total resolution of fatigue and depression.' Results from these studies suggested that IMT can help with more than just gastrointestinal symptoms.

2. Microbiota therapy for ASD

One landmark clinical trial involved the use of oral vancomycin for treating chronic gut problems in children with ASD [23]. Vancomycin is a non-absorbable antibiotic with broad activity against the dominant phyla of obligate anaerobes (Bacteroidetes and Firmicutes) in the intestine [24]. Ten children in this study were treated with vancomycin for 8 weeks, during which the patients reported substantial improvements in both GI and ASD symptoms. However, at the end of treatment, the GI and ASD benefits were lost within a few weeks, even though some children were given standard probiotics. While this study suggested that vancomycin was able to temporarily reduce levels of harmful bacteria possibly causing GI and ASD symptoms, it also showed that antibiotics alone could not permanently reverse the dysbiosis. The long-term failure of vancomycin by itself may be due to its inability to eradicate spores, its detrimental effect on commensal bacteria, and/or the absence of essential microbes to establish new microbial communities and environmental conditions that are more beneficial to the hosts.

An alternative treatment approach is the use of IMT. One study demonstrated substantial long-term benefit of IMT for patients with chronic constipation [25], which is the most common GI problem in ASD. A similar, but much more intensive, approach was used for children with ASD [26]. The treatment involved the use of vancomycin to suppress potentially detrimental bacteria, followed by long-term treatment with cultured intestinal bacteria, including eight Bacteroidetes, eight clostridia, and one *E. coli*. The cultures were prepared overnight from the same frozen stock aliquot at −80°C with approximately 10^9 cells that was ingested twice daily. Cultured microbiota were then mixed in a chocolate milk drink to mask the taste and improve palatability. The children were given pantoprazole (an acid blocker) each morning to allow the bacteria to pass through the stomach with reduced acid load before reaching the bowel. Nine children with ASD and chronic gut problems were treated. At baseline, the children had significant constipation, diarrhea, or alternated between the two symptoms. After 3 months of receiving daily cultured microbiota, there was a substantial improvement in bowel function, and the parents noticed a substantial reduction in the odor of the children's stool. Several children who had abdominal cramps that disrupted their sleep prior to the study experienced a reduction in abdominal cramps by the end of the study and were able to sleep throughout the night. Parents reported marked improvement in vocabulary, task performance, and ability to listen to parents' requests. Some children who initially did not exhibit empathy became emotionally responsive by the end of the study (for example, when they saw another child weeping). Overall, this clinical experience suggested that the GI symptoms in children with ASD are treatable, but that intensive, long-term administration with more diverse microbiota is required.

Based on the initial success of using long-term intensive microbiota transplantation for children with ASD [26], a formal clinical trial of microbiota transplant was conducted for children with ASD [27]. This study treated 18 children with ASD who also had chronic GI disorders. Similar to the initial protocol, the open-label study involved using a combination of oral vancomycin for 14 days, a bowel cleanse to remove bacteria and the vancomycin, a gastric acid reducer to increase the viability of bacteria as they transit through the stomach to the intestine, and the addition of intestinal microbiota (high dose for 1–2 days, and then a lower maintenance dose given daily for 7–8 weeks). Each recipient received microbiota from two different donors, to maximize their exposure to diverse microbiota from healthy donors. This intensive, long-term therapy was termed Microbiota Transfer Therapy (MTT). The MTT was generally safe and well-tolerated with only minor temporary adverse effects due primarily to the vancomycin. Remarkably, MTT resulted in an approximately 80% reduction of GI symptoms, and a 25% reduction of ASD symptoms by the end of treatment. A follow-up evaluation was conducted approximately 2 years after MTT ended (i.e., no additional treatment), and found that the GI improvements were mostly maintained (59% reduction compared to pre-treatment), and the ASD symptoms had continued to improve (47% reduction compared to baseline) [28]. Microbial diversity, which at baseline was significantly lower than that of typically-developing children, increased after treatment, and at 2 years after treatment the diversity remained similar to that for typically-developing children, suggesting that benefits may last for many years. The structure of the gut microbial community changed with treatment, specifically, there were significant increases in the relative abundances of two commensal bacterial genera, *Bifidobacteria* (fivefold increase) and *Prevotella* (84-fold increase) at the two-year follow-up. The fact that improvements to the gut microbiome remained 2 years after treatment and that microbial diversity increased even more, suggest that this treatment was successful in changing the gut environment in a way that it became an environment appropriate to harness beneficial microbes.

However, the level of taxonomic classification for certain bacteria is limited when short reads including conserved regions such as the V4 region of bacterial 16S rRNA gene are targeted for amplicon sequencing, which was a limitation of the Kang et al. 2017 and 2019 studies [27,28]. Previous studies [29–35] have used other methods, including culturing, deep metagenomic shotgun sequencing, microarrays, and quantitative real-time PCR (QPCR) to investigate relative and quantitative levels of specific microbiota in detail. These studies reported that children with ASD typically have relatively increased levels of harmful *Clostridium*, such as *C. histolyticum, C. boltaei*, and *C. perfringens* compared to controls. It would be very useful in future studies to use methods that allow higher taxonomic resolution to determine which microbial species in individuals with ASD are altered by MTT. This strategy may allow development of more targeted therapies. Additionally, a richer understanding of the microbiota may lead to microbial markers to guide diagnosis and treatment of gastrointestinal disorders in autism [36].

Data gathered during the study with MTT [27] also suggested a number of factors that may have contributed to abnormal

development and maturation of gut microbiota, and hence gut problems in the children with ASD. Relative to healthy and typically-developing children, those with ASD and GI problems in the study had a higher rate of C-section births, shorter duration of breastfeeding, higher use of oral antibiotics during infancy, and lower maternal and child fiber consumption. It is worth noting that all of the 18 participants (aged 7–16 years) were reported by their parents to have had GI problems since infancy, and that those GI problems were generally resistant to standard treatment (laxatives, stool softeners, rectal enemas, etc.). Thus, it seems likely that various environmental factors contributed to disruption of normal development of gut microbiota during infancy, and hence were likely contributors to their GI disorders.

It is important to note that this prospective study involved an estimation of the dosing and duration of MTT. Unlike IMT for *C. difficile* infections, which results in rapid improvement of symptoms within a few days and cure of the *C. difficile* infection, it took about 5–6 weeks of MTT for GI symptoms to reach near maximum benefit, and about 10 weeks for maximum benefit. Since a few study participants lost benefit for GI symptoms over the 2 years after treatment started, higher dose of microbiota and/or longer duration with maintenance doses may be helpful, and possibly additional booster doses may be required in some cases.

The pilot study involved pre-treatment with vancomycin, and earlier work [23] had shown that vancomycin alone was temporarily effective in reducing ASD and GI symptoms. However, it is unclear if vancomycin is required when combined with intensive IMT. A disadvantage of vancomycin is that it is nonselective with respect to potentially beneficial or harmful bacteria. Future research is needed to compare MTT with and without pre-treatment with vancomycin, and if needed, to investigate the possible use of more targeted antibiotics.

Importantly, IMT is not risk-free. Just as in any organ or tissue transplant procedure, there is a risk of introducing harmful microbes. In June 2019 the FDA reported that two immunocompromised patients with *C. difficile* infection had received IMT from a donor-harboring multi-drug resistant bacteria containing extended spectrum beta-lactamases, resulting in severe illness and one death [37]. The donor microbiota needs to be rigorously tested for various pathogens and multi-drug resistant organisms. Therefore, it is important to take into consideration the state of the patient's immune system in evaluating the risk of MTT and determination of the optimal treatment regimens. In addition, given that the intestinal microbiota are integral to many aspects of host physiology, it is important to incorporate donor selection criteria that extend beyond the risk of infectious disease, for example, metabolic health and cancer risk.

Because of the promising initial results of MTT for ASD [27,28], in 2019 the FDA granted 'fast-track' status to the use of microbiota transplant therapy for ASD, indicating that it is a promising (not proven) therapy for an unmet need. Given the complexity of the treatment material, it is important that the manufacturing of microbiota-based therapeutics, including IMT, is standardized and development of such products is coupled with rigorous mechanistic research. Furthermore, there is a need for a better understanding of the microbiota in children with ASD, and to search for microbial markers to guide diagnosis and treatment.

Other therapies targeting the microbiota and treating GI disorders in ASD are also being investigated. Although celiac disease is rare in children with ASD [38], gluten-free diets are a popular dietary intervention for autism. Gluten-free diets are often combined with dairy-free diets, since several studies have reported low levels of lactase and other digestive enzymes in children with ASD [39]. However, the evidence of the possible benefit of gluten-free, dairy-free diets for ASD is mixed [4,40]. Another diet that may be useful to consider for patients with ASD is a low Fermentable Oligo-, Di-, Mono-saccharides and Polyols (FODMAP) diet, which has been demonstrated to have benefit in treating irritable bowel syndrome [41,42].

In summary, IMT appears to be a promising treatment for children with ASD and GI symptoms, and worthy of future study. The recent work involved an open-label study with a long-term follow-up, and currently randomized, double-blind, placebo-controlled studies are underway by our group.

3. Expert opinion

Intestinal microbiota transplantation is highly effective for treating *C. difficile* infection and has had limited success for treating other GI disorders. The FDA allows IMT to be used only for treating recurrent *C. difficile* infections, and treatment for other conditions is only allowed as part of a research study with FDA and IRB approval. An open-label study of MTT for gastrointestinal problems in children with ASD included 14 days pre-treatment with vancomycin, a bowel cleanse, and 7–8 weeks of daily intestinal microbiota transplant [27]. This treatment led to a major reduction in GI symptoms, a significant improvement in ASD-related symptoms, and a restoration of normal microbiome diversity. A follow-up study at 2-years post-treatment revealed that most GI benefits remained, ASD symptoms had continued to improve, and microbiome diversity and potentially beneficial microbes had increased further [28].

Overall, the open-label study and a two-year follow-up suggest that intensive MTT is relatively safe and effective in significantly reducing gastrointestinal disorders and autism symptoms, changing the gut microbiota community structure, and increasing the gut microbial diversity. Further research with larger, randomized, double-blind, placebo-controlled studies is warranted, and if the results are similar and consistent, then this MTT may become an important clinical treatment for gastrointestinal disorders in ASD.

There is still a need to optimize the dose and duration of MTT, and to determine if additional doses may be needed in some cases. Also, it may be possible to improve donor selection and/or microbiota product composition. This requires a more in-depth characterization of the microbiome of children with ASD and the effect of MTT on that microbiome. It would also be useful to determine if there are particular biomarkers or indicators that can be used to predict which individuals are most likely to be good candidates for treatment with MTT and the potential need to use repeated doses.

Future work may involve tailoring the composition of the microbiome product, such as by specific donor selection, addition of specific bacteria, or development of specific compositions

of individually cultured bacteria. It is possible that different medical conditions may benefit more from microbiota compositions tailored for each medical condition. There is also a need to develop careful screening procedures, to prevent the introduction of potentially pathogenic bacteria.

Finally, it is unclear if pre-treatment with vancomycin is needed, or needs to be modified in terms of dosing and/or duration. More targeted antibiotics may be more beneficial, due to the detrimental effects of vancomycin on many potentially beneficial bacteria.

Longer-term, it may be possible to substitute particular combinations of bacteria species grown in culture instead of relying on microbiota donations from healthy humans. However, the human intestinal microbiome is extremely complex, and the interactions among microbial groups and the host are complex, making this approach a formidable task.

In summary, within 5 to 10 years we anticipate that MTT may become commercially available for treating gastrointestinal disorders in children and adults with ASD, with additional development of optimized microbiota over the following 5 to 10 years.

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Declaration of interest

Several authors (JBA, D-WK, RKB, TB, AK, and MJS) have pending/approved patents related to the use of IMT and/or probiotics for various conditions including autism. MJS, AK, JBA, RKB, and D-WK have previously received research funding from Crestovo and Finch Therapeutics for IMT research, and JBA and RKB have current research projects with Finch Therapeutics. JBA and RKB were part-time consultants for Crestovo, and are currently part-time consultants for Finch Therapeutics. MJS was previously a part-time consultant for Finch Therapeutics.

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