CHAPTER 8

Serial microbiota analysis after fecal microbiota transplantation in a child with Down syndrome

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ABSTRACT

Fecal microbiota transplantation (FMT) is a very effective treatment for recurrent *Clostridium difficile* infection (CDI) in adults. However, there is a paucity of data on FMT in children and associated microbiome changes in this specific group. We describe a child with Down syndrome and intracranial malignancy, who received FMT for recurrent CDI. Detailed microbiota analysis prior to and following FMT, and pre- and post-recurrence, linked to microbial communities in the donor feces showed that the patient developed a unique microbiota profile after FMT which was very stable over time despite CDI recurrence and subsequent fidaxomicin therapy. *Bacteroidetes* were stably acquired from donor feces, while *Firmicutes*, *Actinobacteria*, *Fusobacteria*, *Verrucomicrobia* and *Proteobacteria* were unique to the patient. The diversity of microbiota of our patient increased from a Shannon diversity index of 2.08 pre-FMT to 3.12 post-FMT.

Conclusion

This is the first description of a child with Down syndrome treated with FMT for recurrent CDI. Our findings indicate that patients with Down syndrome with an alternate immune system, may well tolerate and benefit from FMT even in severe immunocompromised state.

INTRODUCTION

Clostridium difficile is an obligate anaerobic, Gram-positive, spore-forming bacillus, and the most frequent cause of healthcare associated diarrhea. In the last ten years, the rate of pediatric hospitalization with CDI has nearly doubled.[1,2] Infection commonly results from prolonged administration of antibiotic therapies which disrupts the intestinal microbiota composition, commonly characterized by low microbial diversity, facilitating colonization with *C. difficile*.[3] Current treatment protocols recommend metronidazole (30 mg/kg/day for 10 days) as initial treatment option for pediatric CDI. However, over 20% of the pediatric patients with a first episode of CDI develop a recurrent infection.[4,5] Risk factors for the development of recurrent CDI in children include underlying inflammatory bowel disease, malignancy, recent surgery, and the number of antibiotic exposures by class.[4,6-8] In particular the presence of severe comorbidity is an important risk factor for the development of primary and recurrent CDI.[4,9] Fecal microbiota transplantation (FMT) has been proven a safe and effective treatment for recurrent CDI in adult subjects and children.[8,10-12] However, pediatric studies are limited in sample size.[8] Effects of FMT rely on restoration of patients' disturbed intestinal microbiota by the high diversity of microbial communities present in donor feces.[13,14] In this case report, we describe a 14 years old child with Down syndrome and intracranial malignancy, with recurrent CDI treated with FMT. Down syndrome has been associated with various immunological impairments, and abnormalities in function of both innate and adaptive immunity, may lead to diminished viral and bacterial clearance.[15,16] To our knowledge, this is the first child with Down syndrome treated with FMT for recurrent CDI. Furthermore, we describe the impact of FMT on the gut microbiota composition, prior to and following FMT, linked to microbial communities in the donor feces.

CASE PRESENTATION

A 14-year old girl was referred for FMT because of recurrent CDI. Her medical history revealed Down syndrome, and a recently diagnosed choroid plexus carcinoma. This choroid plexus carcinoma was complicated by hydrocephalus, trans-ependymal leakage of cerebrospinal fluid and vasogenic oedema in the left cerebral hemisphere, treated with dexamethasone and an external ventricular drain, which was internalised after two weeks. Total surgical resection of the choroid plexus carcinoma was performed, and no neurological impairments were observed following this intervention. Postoperatively, patient was treated according to the CPT 2001 protocol arm A, including alternating cycles of

cyclophosphamide/etoposide/vincristine, and carboplatin/etoposide/vincristine. A magnetic resonance imaging scan performed after two treatment cycles showed continued complete response. After two cycles of chemotherapy, cranio-spinal radiotherapy was administered for a period of six weeks. During this period, she was treated with ciprofloxacin because of a pharyngeal infection with *Pseudomonas aeruginosa* and trimethoprim-sulfamethoxazole was prescribed as *Pneumocystis jirovecii* pneumonia prophylaxis. At this stage, the patient developed foul-smelling diarrhea up to four times per day. Stool tests for *Salmonella*, *Shigella*, *Yersinia*, *Campylobacter*, parasites and adeno- and rotavirus were all negative. After one month of diarrhea, these tests were repeated and extended with toxigenic culture for *C. difficile*, which was positive. This first episode of CDI was treated with a 14-day course of oral metronidazole (500 mg TID). Her gastrointestinal complaints resolved completely during this course but recurred several days after cessation of CDI therapy (*C. difficile* toxin positive), and she was treated again with a 14-day course of metronidazole (500 mg TID). Again, watery diarrhea resolved completely but reoccurred within one week after cessation of antibiotics. Again, *C. difficile* toxin was positive, and the patient was now treated with a 14-day course of oral vancomycin (250 mg TID). Shortly after cessation of vancomycin, patient developed watery diarrhea again (*C. difficile* toxin positive), and treatment with vancomycin was restarted for a prolonged period of four weeks. During all these antibiotic treatment episodes, stools returned to normal and the patients clinical condition improved dramatically. Due to the recurrent/refractory nature of CDI, the ongoing low blood counts, and poor clinical condition (immobility, lack of energy, complete tube feeding dependency) it was decided in consultation with parents to renounce from further chemotherapy, two cycles prior to completing the protocol. Since administration of antibiotics seemed to control but not clear the CDI, and because of the great impact of the infection on her general well-being, FMT as an alternative treatment for recurrent CDI was suggested. The child's parents agreed to this therapy, and informed consent was obtained (including collection of follow-up fecal samples). FMT material was obtained from a healthy, rigorously screened donor from OpenBiome, an international public stool bank. The donorfeces solution was administered via a nasoduodenal tube under general anaesthesia, preceded by five days of oral vancomycin (250 mg TID), and full bowel lavage by means of four litres of polyethylene glycol solution (Kleanprep®). Vancomycin treatment was stopped 24 h prior to the FMT procedure and no medication was prescribed after the FMT procedure. In the first five days following FMT, patient did not pass any stools. In the subsequent two weeks the diarrhea had resolved completely and she passed formed stools twice daily.

Unfortunately, the patient developed diarrhea again 18 days after FMT and cultures were positive for *C. difficile*. Since it has been suggested to treat a first recurrence of CDI after FMT with antibiotics, with a preference for the narrow spectrum antibiotic fidaxomicin,[17] a 10-day course of fidaxomicin 200 mg (BID) was prescribed. With this policy, gastro-intestinal symptoms resolved completely during the follow-up period of 16 weeks; stool consistency normalized and the general well-being of the patient improved to pre-CDI level.

Post-FMT follow up: microbiota analysis

Intestinal microbiota analysis was performed on fecal samples of the patient collected prior to and post-FMT (Figure 8.1): (1) pre-FMT; (2) first sample produced after FMT; (3) CDI recurrence 18 days post-FMT, prior to start of fidaxomicin; (4) last day of fidaxomicin treatment and (5) ten days after cessation of fidaxomicin. Microbiota profiles were compared with microbial communities from the donor sample (6).

Microbiota analysis was performed by IS-pro, a DNA-based microbiota profiling technique, based on the identification of species-specific length polymorphisms of the 16S-23S rDNA interspacer (IS) region, and phylum-specific sequence polymorphisms of 16S rDNA.[18] Measured length of this IS region, displayed by number of nucleotides, corresponds with different bacteria at species level based on a database consisting of over 1500 species and their corresponding IS lengths. Peak height of each IS-pro profile, expressed in relative fluorescence units, corresponds to the quantity of PCR product and reflect the relative abundance of present species.[18] In a recent study, it was shown that intestinal microbiota characterization by IS-pro and 454-pyrosequencing generated comparable results.[19] IS-pro provides results of microbiota analysis within a few hours and is therefore optimized for clinical use of gut microbiota analysis.

Pre-FMT microbiota composition of our patient was characterized by very low diversity (Shannon diversity index 2,08); only a limited number of different species was present in low abundance. It has been shown that antibiotic therapy influences intestinal microbiota composition in children.[20,21] In addition, different types of cancer have been associated with intestinal dysbiosis, depending on underlying disease, mucosal disruption, bowel motility disturbance, medication and enteral/parenteral nutrition.[22] It is likely that both factors, prolonged use of antibiotics and malignancy, contributed to the decreased diversity of the gut microbiota in the presented patient, leading to predisposition to colonization with *C. difficile*. Although information on presence of characteristic microbial signatures

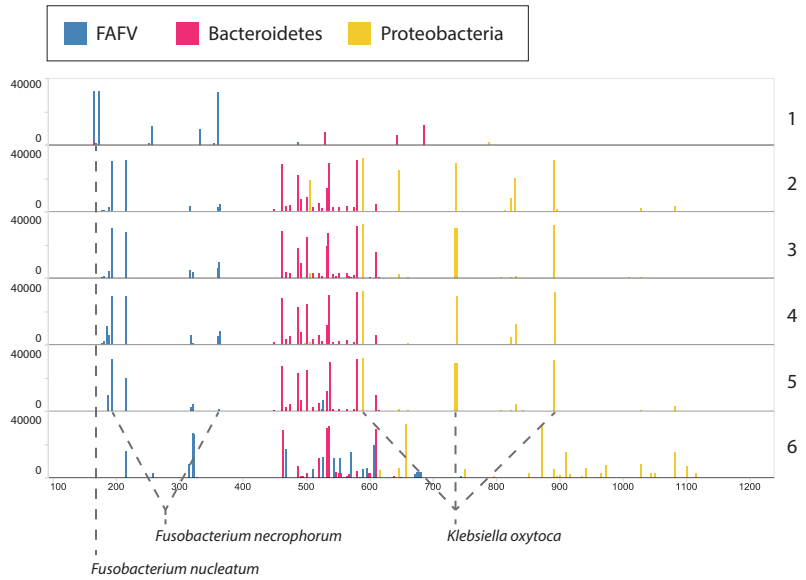

Figure 8.1 Microbiota profiles pre- and post fecal microbiota transplantation.
IS profiles of fecal samples collected pre- and post fecal microbiota transplantation (FMT) compared to donor profile. (1) pre-FMT; (2) first sample post-FMT (5 days after FMT); (3) sample collected during CDI recurrence (18 days post-FMT) prior to start of fidaxomicin; (4) sample on last day of fidaxomicin treatment (5) sample ten days after cessation of fidaxomicin, and (6) IS profile of the donor feces.
Horizontal axis of each profile displays IS fragment length expressed in number of nucleotides, corresponding to bacterial operational taxonomic unit (OTU). Vertical axis of each profile displays the relative abundance of the corresponding OTU. Blue peaks represent *Firmicutes*, *Actinobacteria*, *Fusobacteria*, *Verrucomicrobia* (FAFV), red peaks represent *Bacteroidetes* and yellow peaks represent Proteobacteria.
Microbiota composition of pre-FMT sample is characterized by presence of very limited number of species, a relatively high abundance of *Fusobacterium nucleatum* and almost complete absence of Proteobacteria. The patient developed a unique microbiota profile which was very stable over time despite CDI recurrence and subsequent fidaxomicin therapy. *Bacteroidetes* were stably acquired form donor feces, while FAFV and *Proteobacteria* were unique to the patient. *Clostridium difficile* could be detected in the patients profiles prior to fidaxomicin therapy, but not afterwards (abundance too low to be seen in this figure).

in Down syndrome is limited, it has been shown that the gut microbiota in this specific population is largely dominated by the phyla *Firmicutes*, *Actinobacteria*, and *Bacteroidetes*.[23] *Proteobacteria*, *Verrucomicrobia* and *Fusobacteria* represent the more subdominant phyla.[23] In our patient, *Fusobacterium nucleatum* was observed in relatively high abundance and *Proteobacteria* were almost completely absent pre-FMT.

A sample taken five days post FMT (first stool) showed a marked increase in diversity (Shannon diversity index 3,12) compared to pre-FMT, which was mainly attributable to

species from the phylum *Bacteroidetes*. The transfer of the phylum *Bacteroidetes* was highly efficient: almost all *Bacteroidetes* species present in the donor feces were also found in the recipient five days after FMT and remained present in all following time points. Remarkably, hardly any of the donor species from the phyla *Firmicutes* and *Proteobacteria* could be detected in the recipient, while a high abundance of *Fusobacterium necrophorum* and *Klebsiella oxytoca* were found in the recipient five days after FMT, both of which species were not detected preceding transplantation in the recipient or in the donor feces. *C. difficile* could still be detected in low abundance after FMT. Post-FMT course was characterized by a very stable course over time of species within all phyla, despite occurrence of a CDI recurrence and treatment with fidaxomicin. Interestingly, after treatment with fidaxomicin, *C. difficile* was no longer detected, while further microbiota composition remained unchanged.

Microbiota and immune system of children with Down syndrome

Knowledge on characteristics of intestinal microbiota composition in Down syndrome is very limited. The largest study on this topic comprised the comparison between the gut microbiota structure of 17 adults with Down syndrome with that of 16 healthy, non-trisomy controls by means of 454 pyrosequencing.[23] Comparable levels of microbial diversity and a similar overall composition was observed in both groups. However, in-depth analysis showed that microbiota of subjects with Down syndrome was characterized by a significant higher abundance in several subdominant genera, including *Parasporobacterium* and *Sutterella*, as well as by a reduction in the abundance of *Veillonellaceae*. Current knowledge on the impact of potential differences in microbial signatures of subjects with Down syndrome is obviously too limited to draw firm conclusions and to adapt FMT-related strategies in patients with Down syndrome. Possibly more important, Down syndrome has been associated with various immunological impairments, linked to an increased risk for leukemia and auto-immune diseases.[15] Furthermore, abnormalities in function of both innate and adaptive immunity may lead to diminished viral and bacterial clearance.[16] The impaired immunological function in these patients may explain the recurrent nature of CDI in our case description. Previous case series on FMT in children included several patients receiving simultaneous immunosuppression who tolerated FMT well, indicating that an immunocompromised state is not an absolute contraindication for application of FMT in children.[8]

Fecal Microbiota Transplantation in children

It is suggested that recurrent CDI is caused by the incomplete recovery of the gut microbiota after antibiotic treatment against CDI. FMT restores the disrupted gut microbiota, leading to colonization resistance preventing germination of *C. difficile* spores and has gained increasing attention with its outstanding efficacy in treating recurrent CDI in adults. However, data about the effectiveness in children are still scarce. To date, only a few case-reports and case-series of children treated with FMT have been described.[13,14,24-28] These studies have shown that FMT, both via colonoscopy and via nasoduodenal tube, seems to be safe, well tolerated, and effective for pediatric patients with recurrent CDI, with response rates up to 95%. Nicholson et al. identified malignancy as the most important risk factor in children for both primary and recurrent CDI.[4] They also described an insufficient host immune response to be a causal risk factor for development of recurrent CDI. The patient described here fulfilled all these criteria; the combination of underlying Down syndrome, choroid plexus carcinoma, and treatment with chemotherapy and antibiotics, have possibly contributed to the occurrence of multiple CDI recurrences. Development of a post-FMT recurrence could possibly also be directed to this extensive underlying comorbidity. It has been suggested that patients who develop a post-FMT CDI recurrence have at least a partially restored gut microbiota, which is reflected by an increased efficacy of antibiotic treatment for CDI compared to the pre-FMT state.[17] In our patient, post-FMT recurrence was successfully treated with fidaxomicin, which supports this hypothesis. We preferred fidaxomicin over vancomycin, despite higher costs, because it has been shown that fidaxomicin has less negative influence on the precarious balance of the gut microbiota compared to vancomycin, sparing commensal microbiota and thus leading to a lower risk of recurrence.[29] This is supported by our data, where fidaxomicin did not influence the composition of the gut microbiota (Figure 8.1).

Although previous studies have suggested that FMT is a viable option that could circumvent children from adverse events and complications associated with indefinite use of metronidazole or vancomycin, there are still several important aspects that need to be addressed in future studies regarding safety and efficacy of FMT in children. These include selection of the optimal donor (adult or pediatric), assessment of the optimal way of administration of donor material (duodenal or colonic infusion), and evaluation of assumed benefits of pre-FMT preparation with antibiotics and laxatives.

CONCLUSION

In conclusion, we have described the first case of successful FMT for recurrent CDI in a child with Down syndrome. Our findings underscore that children with Down syndrome, characterized by an alternate immune system, may well tolerate and benefit from FMT, even in severe immunocompromised state due to comorbid malignancy.

REFERENCES

1. Kim J, Smathers SA, Prasad P, Leckerman KH, Coffin S, Zaoutis T (2008) Epidemiological features of Clostridium difficile-associated disease among inpatients at children's hospitals in the United States, 2001-2006. Pediatrics 122:1266-1270
2. Sammons JS, Toltzis P (2013) Recent trends in the epidemiology and treatment of C. difficile infection in children. Current opinion in pediatrics 25:116-121
3. Lo Vecchio A, Zacur GM (2012) Clostridium difficile infection: an update on epidemiology, risk factors, and therapeutic options. Current opinion in gastroenterology 28:1-9
4. Nicholson MR, Thomsen IP, Slaughter JC, Creech CB, Edwards KM (2015) Novel risk factors for recurrent Clostridium difficile infection in children. Journal of pediatric gastroenterology and nutrition 60:18-22
5. Morinville V, McDonald J (2005) Clostridium difficile-associated diarrhea in 200 Canadian children. Can J Gastroenterol 19:497-501
6. Hourigan SK, Oliva-Hemker M, Hutfless S (2014) The prevalence of Clostridium difficile infection in pediatric and adult patients with inflammatory bowel disease. Digestive diseases and sciences 59:2222-2227
7. Kelsen JR, Kim J, Latta D, Smathers S, McGowan KL, Zaoutis T, Mamula P, Baldassano RN (2011) Recurrence rate of clostridium difficile infection in hospitalized pediatric patients with inflammatory bowel disease. Inflammatory bowel diseases 17:50-55
8. Hourigan SK, Oliva-Hemker M (2016) Fecal microbiota transplantation in children: a brief review. Pediatr Res
9. Guarino A, Ashkenazi S, Gendrel D, Lo Vecchio A, Shamir R, Szajewska H, European Society for Pediatric Gastroenterology H, Nutrition, European Society for Pediatric Infectious D (2014) European Society for Pediatric Gastroenterology, Hepatology, and Nutrition/European Society for Pediatric Infectious Diseases evidence-based guidelines for the management of acute gastroenteritis in children in Europe: update 2014. Journal of pediatric gastroenterology and nutrition 59:132-152
10. van Nood E, Vrieze A, Nieuwdorp M, Fuentes S, Zoetendal EG, de Vos WM, Visser CE, Kuijper EJ, Bartelsman JFWM, Tijssen JGP, Speelman P, Dijkgraaf MGW, Keller JJ (2013) Duodenal infusion of donor feces for recurrent Clostridium difficile. N Engl J Med 368:407-415
11. Cammarota G, Ianiro G, Gasbarrini A (2014) Fecal microbiota transplantation for the treatment of Clostridium difficile infection: a systematic review. Journal of clinical gastroenterology 48:693-702.

12. Surawicz CM, Brandt LJ, Binion DG, Ananthakrishnan AN, Curry SR, Gilligan PH, McFarland LV, Mellow M, Zuckerbraun BS (2013) Guidelines for diagnosis, treatment, and prevention of clostridium difficile infections. American Journal of Gastroenterology 108:478-498

13. Hourigan SK, Chen LA, Grigoryan Z, Laroche G, Weidner M, Sears CL, Oliva-Hemker M (2015) Microbiome changes associated with sustained eradication of Clostridium difficile after single faecal microbiota transplantation in children with and without inflammatory bowel disease. Alimentary pharmacology & therapeutics 42:741-752

14. Walia R, Garg S, Song Y, Girotra M, Cuffari C, Fricke WF, Dutta SK (2014) Efficacy of fecal microbiota transplantation in 2 children with recurrent Clostridium difficile infection and its impact on their growth and gut microbiome. Journal of pediatric gastroenterology and nutrition 59:565-570

15. Goldacre MJ, Wotton CJ, Seagroatt V, Yeates D (2004) Cancers and immune related diseases associated with Down's syndrome: a record linkage study. Arch Dis Child 89:1014-1017

16. Cocchi G, Mastrocola M, Capelli M, Bastelli A, Vitali F, Corvaglia L (2007) Immunological patterns in young children with Down syndrome: is there a temporal trend? Acta Paediatr 96:1479-1482

17. van Beurden YH, de Groot P, Van Nood E, Nieuwdorp M, Keller JJ, Goorhuis A (2016) Complications, effectiveness, and long term follow-up of fecal microbiota transfer by nasoduodenal tube for treatment of recurrent Clostridium difficile infection. UEG journal DOI: 10.1177/2050640616678099

18. Budding AE, Grasman ME, Lin F, Bogaards JA, Soeltan-Kaersenhout DJ, Vandenbroucke-Grauls CMJE, van Bodegraven AA, Savelkoul PHM (2010) IS-pro: high-throughput molecular fingerprinting of the intestinal microbiota. FASEB J 24:4556-4564

19. de Meij TG, Budding AE, de Groot EF, Jansen FM, Frank Kneepkens CM, Benninga MA, Penders J, van Bodegraven AA, Savelkoul PH (2015) Composition and stability of intestinal microbiota of healthy children within a Dutch population. FASEB J

20. Fernandes MR, Ignacio A, Rodrigues VA, Groppo FC, Cardoso AL, Avila-Campos MJ, Nakano V (2016) Alterations of Intestinal Microbiome by Antibiotic Therapy in Hospitalized Children. Microb Drug Resist

21. Korpela K, Salonen A, Virta LJ, Kekkonen RA, Forslund K, Bork P, de Vos WM (2016) Intestinal microbiome is related to lifetime antibiotic use in Finnish pre-school children. Nature communications 7:10410

22. Castagnola E, Ruberto E, Guarino A (2016) Gastrointestinal and liver infections in children undergoing antineoplastic chemotherapy in the years 2000. World journal of gastroenterology 22:5853-5866

23. Biagi E, Candela M, Centanni M, Consolandi C, Rampelli S, Turroni S, Severgnini M, Peano C, Ghezzo A, Scurti M, Salvioli S, Franceschi C, Brigidi P (2014) Gut microbiome in Down syndrome. PloS one 9:e112023

24. Russell G, Kaplan J, Ferraro M, Michelow IC (2010) Fecal bacteriotherapy for relapsing Clostridium difficile infection in a child: a proposed treatment protocol. Pediatrics 126:e239-242

25. Kahn SA, Young S, Rubin DT (2012) Colonoscopic fecal microbiota transplant for recurrent Clostridium difficile infection in a child. The American journal of gastroenterology 107:1930-1931

26. Russell GH, Kaplan JL, Youngster I, Baril-Dore M, Schindelar L, Hohmann E, Winter HS (2014) Fecal transplant for recurrent Clostridium difficile infection in children with and without inflammatory bowel disease. Journal of pediatric gastroenterology and nutrition 58:588-592

27. Pierog A, Mencin A, Reilly NR (2014) Fecal microbiota transplantation in children with recurrent Clostridium difficile infection. The Pediatric infectious disease journal 33:1198-1200

28. Kronman MP, Nielson HJ, Adler AL, Giefer MJ, Wahbeh G, Singh N, Zerr DM, Suskind DL (2015) Fecal microbiota transplantation via nasogastric tube for recurrent clostridium difficile infection in pediatric patients. Journal of pediatric gastroenterology and nutrition 60:23-26

29. Louie T, Cannon K, Denis MS, Byrne B, Ward L (2010) Quantitative real-time PCR measurement of the impact of fidaxomicin or vancomycin treatment of Clostridium difficile infection on the intestinal microbiome, compared with normal controls. Clinical Microbiology and Infection 16:S166