
L. Ward1,2, H. O'Grady1,2, K. Wu1, M. Workentine2, T. Louie1,3

Cumming School of Medicine1 and Faculty of Veterinary Medicine2, University of Calgary and Alberta Health Services3 Calgary, Canada

Case#  Home  Sex/ Age of ASD (yr)  Clinical course
1  USA  F/4.5  3.8  LPA  Complete symptom resolution, recovered partially on antibiotic exposure 4 months later, re-FMT with received ASD features marked improvement, regression with LPA antibiotic exposure, partial remission
2  CAN  F/2  1.5  LPA  C. innocuum/Klebsiella pneumoniae 146, >10^7/g  Marked improvement, social, facotrs, multi score, improved but LPA evaluation difficult
3  USA  M/12  2.5  LPA  C. innocuum/Klebsiella pneumoniae 146, >10^7/g  Marked improvement, partial remission
4  USA  F/2  1  LPA  C. innocuum/Klebsiella pneumoniae 146, >10^7/g  Marked improvement, partial remission
5  USA  M/12  19  LPA  C. innocuum/Klebsiella pneumoniae 146, >10^7/g  No change, LPA
6  CAN  M/12  6  LPA  No notable recovery  Market improvement ≥ 2 months, regression with antibiotic use, improved with re-FMT
7  CAN  M/12  5  LPA  Cultures not done  Moderate temporary improvement ≤ 3 weeks, regression reappears
8  USA  F/1.8  2  LPA  Cultures not done  Improvement was sustained with longer duration of microbial administration, follow up continues
9  CANNUSA  M/2.5  6.5  LPA  C. innocuum/Klebsiella pneumoniae 146, >10^7/g  Improved social function, early evacuation

Introduction:
- Autism Spectrum Disorder (ASD), a developmental neuro-psychiatric disability mainly recognized at ~ 2 years of age now affects 1 in 68 children aged up to 8 years (MMWR April 1, 2016).
- Genetic and environmental factors, in combination are suspected. A gut-brain axis postulates that intestinal microbes can alter or modify immune function and influence neurodevelopment in a bi-directional manner.
- In a cohort of autistic children, vancomycin treatment temporarily improve the clinical severity of ASD behavior (Sandler, J Clin Neurol 2000; 15: 429-35). Since vancomycin impairs the normal gut microbiota, primarily Firmicutes and Bacteroidetes, it is hypothesized that FMT might result in more durable clinical outcomes. Song and Fingold demonstrated that a clostridial species in the C. clostridioforme group is associated with late onset ASD.
- Reported here, in part, is a small case series of patients who their parents sought services in a clinical practice setting to explore if FMT would be of benefit. A prior, based on antibiotic suppression and post FMT fecal sample testing strategy was employed to determine if microbial differences could be observed.

Methods:
- Between June 2011 and April 2016 the parents of this cohort of subjects enquired directly or were referred by their care provider as to whether an FMT would be accessible. Symptoms/signs of ASD were assessed by the Childhood Autism Rating Scale criteria.
- Serial fecal samples prior to FMT, after completion of suppression of possible ASD-associated microbes and after FMT were collected at 1, 3 months and if the clinical course changed.
- The oral ‘conditioning’ regimens used were vancomycin 40 mg/kg in oral BID dosage x7 days, or vancomycin plus added nitazoxanide 200 mg BID, colistin as colymin M 37 mg BID and nystatin 500000 unit TID each x 7 days (2016 for 3 patients).
- After a magnesium citrate bowel prep to evacuate intestinal contents, oral FMT with fecal capsules (n=12-24) containing 0.47 ml of pelleted fecal microbes obtained from screened adult donors (2 from a parent) followed by 300-400 ml of a fecal slurry made from 50-100 gm stooled fluid instilled by enema, followed by a repeat administration of fecal capsules the next morning.
- Fecal samples in 5 gm neat and 2.5 gm aliquots in 2.5 ml of BHIG broth both were frozen at -800 C
- For selected patients, quantitative fecal cultures diluted samples 10-3,7,10 onto Fastidious Anaerobic Agar, BAP and Colistin Nalidixic Acid Columbia Blood Agar for recovery of most numerous Gram-positive obligate anaerobes. Unknowns were streaked for purity, disc ID with kanamycin, vancomycin and colistin discs, and subsequently underwent DNA extraction, purification, and PCR amplification of the RNA gene. Product was sent to Macrogen,Inc, Seoul, Korea for sequencing following GenBank assignment of potential identity.
- Follow DNA extraction and purification of 0.2 g fecal samples, Illumina sequencing of the V3 and V4 regions of the 16s RNA gene was performed to assess differences in microbial composition prior to FMT, prior to FMT but after 7 days of antibiotic exposure, and following FMT.