A Case Study of the Gut Microbiome in ASD: Correlation of Microbial Profiles with GI and Behavioral Symptoms

R. A. Luna1,2, A. Magee1,2, J. K. Runge1,2, A. Venkatachalam, M. RubioGonzales 1,2 and J. Versalovic 1,2
(1)Texas Children's Microbiome Center, Texas Children's Hospital, Houston, TX, 2)Department of Pathology & Immunology, Baylor College of Medicine, Houston, TX

Background: Gastrointestinal (GI) disorders are now widely recognized as a clinical symptom of autism spectrum disorder, and research into the microbiome-gut-brain axis is beginning to reveal the interconnectivity between GI pain and potential behavioral challenges. Large studies in well-controlled cohorts are needed to provide the baseline dataset for this population, but there can be much learned from individual case studies, especially those with a longitudinal component. As a prelude to a much larger study underway, a single child with autism was followed over a 2-week period, including daily stool collection, alongside a neurotypical sibling for comparison.

Objectives: The objectives of this case study were to 1) evaluate current sequencing and analysis strategies to assess the microbial profile in a child with autism compared to an unaffected sibling, 2) correlate GI symptoms, stooling pattern, and diet diaries as well as available behavioral data over a 2-week period, and 3) identify organisms of interest for exploration in a larger dataset in the future.

Methods: Over a 2 week period, daily stool samples (plus one additional sample) were collected from the child with ASD with a single stool sample collected from an unaffected sibling. Next-generation sequencing (454 and Illumina) of the V1V3 and V4 variable regions of the 16S rRNA gene was performed, and resulting sequences were quality filtered, assigned to operational taxonomic units (OTUs), and classified based on comparison to the Greengenes database. Individual species of interest in ASD as well as OTUs of significance related to the child with ASD and absent in the unaffected sibling were identified.

Results: Principal component analysis revealed clear separation between the 15 samples obtained from the child with ASD compared to the unaffected sibling. Three organisms (individual OTUs) previously reported in autism were identified in the microbial profile of each of the 15 ASD samples but absent in the microbial profile of the unaffected sibling: *Sarcina ventriculi*, *Barnesiella intestihominis*, and *Clostridium bartlettii*. In addition, distinct differences were seen in the microbiome of the ASD child during days 6-8, a period where GI symptoms were reported (pain and diarrhea). Of note, the detection of *Haemophilus parainfluenzae* appeared to coincide with the onset of the GI episode. Beyond the correlation of GI symptoms with changes in the microbiome, that period also coincided with a trend toward behavior challenges, specifically an increase in self-injurious behavior. A separate 2-day period exhibiting

the same microbial profile did not coincide with a change in GI symptoms but was accompanied by the same increase in self-injurious behavior, suggesting the possibility of GI pain that was not reflected in a change in stooling pattern.

Conclusions: While in-depth studies of the gut microbiome in autism that are underway will generate the critical mass of data needed as a comparison dataset, this case study illustrates the benefit of longitudinal sampling of a single patient in parallel with collection of clinical metadata (behavioral, gastrointestinal, and dietary). Microbiome characterization shows great potential as a companion diagnostic in the evaluation of individuals with autism spectrum disorder.