A Microbial Association with Autism

Jorge L. Benach, Ellen Li, and Margaret M. McGovern

Departments of Molecular Genetics and Microbiology, Medicine and Pediatrics, Stony Brook University, Stony Brook, New York, USA

ABSTRACT Autism is a heterogeneous group of complex developmental disabilities that result from a number of possible etiologies. There are a well-known number of comorbidities associated with autism spectrum disorders (ASD), including, commonly, gastrointestinal (GI) pathology, which can include variable combinations of constipation, diarrhea, abdominal pain, gastroesophageal reflux, and vomiting. An American Academy of Pediatrics consensus panel has recommended that prospective studies be carried out to determine the prevalence of GI disorders in ASD and their pathophysiologic basis. In a recent article, Williams et al. [B. L. Williams, M. Hornig, T. Parekh, and W. I. Lipkin, mBio 3(1):e00261-11, 2012] have provided one such study of autism with GI comorbidities by presenting evidence of *Sutterella* species in ileal mucosal biopsy specimens from patients diagnosed with ASD but not in control children with GI symptoms, suggesting a specific role for *Sutterella* in ASD. *Sutterella* sequences represented ~1 to 7% of the total bacterial sequences, and this is a very large effect size on the ileal mucosal composition of the autism phenotype, rivaling or perhaps exceeding the effect size of the ileal Crohn's disease phenotype. This study opens a new field of investigation to study the etiology or consequences of GI comorbidities in ASD.

Gastrointestinal (GI) pathology is a common feature of autism spectrum disorders (ASD), which are a heterogeneous group of complex developmental disabilities that result from a variety of etiologies. Understanding the pathophysiology of the GI clinical symptoms in ASD is important for the early identification of GI pathology and for guiding therapy.

The diagnosis of ASD is primarily based upon behavioral criteria, rather than physical examination findings or laboratory tests. Criteria include impaired social interactions, deficits in verbal and nonverbal communication, and repetitive behaviors. The specific diagnostic criteria for ASD are detailed in the American Psychiatric Association's *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV) and define three subgroups of ASD. These include autistic disorder (AD), Asperger syndrome (AS), and pervasive developmental disorder-not otherwise specified (PDD-NOS), or atypical autism (1). Signs of autism may be present in infancy. However, many affected children are not diagnosed until after the second year of life, when they frequently come to medical attention due to language delay. In others, there is a period of essentially normal development followed by regression (2, 3). Medical comorbidities can appear at any age. Over the past several decades, heightened awareness of the signs and symptoms of autism among physicians, parents, and educators has led to documentation of a prevalence rate for ASD as high as 1 in 110 8-year-old children (4). Since there is evidence for a beneficial effect of early intervention in children with ASD, the American Academy of Pediatrics (AAP) recommends screening for autism during scheduled primary care visits, a move that has further contributed to an increased recognition of ASD.

A prominent feature of ASD is poor communication skills that make it difficult for patients to articulate their medical complaints. This is particularly problematic in patients with GI-related illness that can present as nonspecific abdominal pain or as an escalation of disruptive or self-injurious behavior. Therefore, caregivers must have a high index of suspicion to provide prompt and appropriate medical evaluations for GI manifestations.

Given the high prevalence of ASD, it is hardly surprising that considerable attention has been focused on evaluating all possible links that could have an etiological role in autism. Twin and family studies suggest that genetic factors do play an important role in ASD. Progress in identifying these genetic factors has been advanced by the availability of DNA banks that facilitate genetic analyses. Indeed, in approximately 20% of affected patients, cytogenetically visible chromosomal abnormalities and copy number variants are detectable by chromosomal microarray analysis (5). Another 5% of ASD are associated with single-gene disorders. Genome-wide association studies and the investigation of candidate genes are being carried out in numerous research laboratories. These studies have successfully identified a number of neurodevelopmental candidate genes associated with autism (6).

Rightly or wrongly, the etiology of autism has also been associated with vaccination. The public concern over the association of childhood vaccines with autism is certainly subsiding, but dispelling generally accepted dogma, albeit erroneous or fraudulent, is difficult. Hornig et al. (7) found no evidence of measles virus in intestinal tissue samples, making it less likely that a component of the mumps-measles-rubella (MMR) vaccine is linked to autism. In comprehensive reviews, the Institute of Medicine concluded that that no such link exists (8, 9).

Further progress in identifying the causes of ASD may be facilitated by delineating specific phenotypes within this heterogeneous group of patients. However, the identification of such phenotypes has proven to be challenging. These efforts may be aided by the careful medical evaluation of patients with ASD to identify patterns of medical comorbidities. Approaches for such evaluations have been suggested by the American Academy of Pediatrics and the American College of Medical Genetics (10, 11).

Similarly, systematic approaches to evaluation of the medical comorbidities in ASD will permit the identification of patients with GI symptoms. These include variable combinations of con-

Published 14 February 2012

Citation Benach JL, Li E, McGovern MM. 2012. A microbial association with autism. mBio 3(1):e00019-12. doi:10.1128/mBio.00019-12.

Address correspondence to Jorge L. Benach, jorge.benach@stonybrook.edu.

stipation, diarrhea, abdominal pain, gastroesophageal reflux, and vomiting. Indeed, several studies have reported a prevalence of GI symptoms in ASD as high as 70%, although an AAP consensus panel has recommended that prospective studies be carried out to determine the actual prevalence of GI disorders in ASD and their pathophysiologic basis (12). In their publication in *mBio*, Williams et al. (13) have provided one such evaluation of autism with GI comorbidities by presenting evidence of *Sutterella* species in ileal mucosal biopsy specimens from patients diagnosed with ASD but not from control children with GI symptoms, suggesting a specific role for *Sutterella* in ASD.

A number of studies designed to assess the relationship between GI symptoms in ASD patients and the microbiome have already disclosed some interesting trends. The human intestinal microbiome is comprised of a large number of highly diverse commensal bacteria that confer benefit to the host, including, for example, bolstering the function of the immune system in early infancy, facilitating the acquisition of essential nutrients, and regulating human physiological homeostasis. A significant amount of interest has been generated regarding the role of the microbiome as an arbiter of a number of inflammatory and metabolic abnormalities. The use of the term "arbiter" is deliberate since the relationship of the microbiome to "dysbiosis" is only by association, and not as a cause or consequence of diseases. Indeed, metagenomics applied to the microbiome has shown the presence of significant individual variability in intestinal tract microbiome composition and the identification of enterotypes (14, 15).

In patients with ASD, an early study of fecal flora, which was prompted by the use of oral vancomycin, documented an increase in clostridial species in autistic patients compared to controls (16). This finding was confirmed in a second study that also showed a higher incidence of *Clostridium* (clusters I and II) organisms in patients with ASD than in healthy children, including siblings of the autistic group (17). Other investigations of the fecal microbiome in patients with ASD and GI symptoms showed high levels of *Bacteroidetes* in the ASD group, whereas *Firmicutes* were higher in controls (18). Similarly, Adams and coworkers have demonstrated an increase in *Lactobacillus* species and a reduction in *Bifidobacter* organisms in the stools of ASD patients compared to controls (19).

While the previous reports have examined fecal specimens, Williams and coworkers (20) took a new approach and included intestinal gene expression and identification of the microbiota in the intestinal mucoepithelium. Their study revealed deficiencies in the enzymatic activity of disaccharidases and hexose transporters among the ASD patient group. These deficiencies suggest that impaired digestion and transport of intestinal carbohydrates may contribute to ASD GI symptoms. Consistent with the notion that the intestinal microbiota contributes to the enzymatic activities of the human intestine needed to degrade carbohydrates, there were also changes in the microbiota identified among the ASD group. Metagenomic analyses disclosed a significant dysbiosis with reductions in *Bacteroidetes* and increases in the ratio of *Firmicutes* to *Bacteroidetes*, as well as in *Betaproteobacteria*.

Williams et al. (13) report detecting *Sutterella* 16S rRNA gene sequences in ileal mucosal biopsy specimens from 12 of 23 patients diagnosed with autism and GI symptoms but in none of the specimens from 9 control children with GI symptoms. *Sutterella wadsworthensis* sp. nov. was described 15 years ago from clinical isolates from patients with infections that were below the diaphragm (21). In addition to the molecular taxonomy that Williams et al. describe (13), *Sutterella* can be identified fairly easily in the laboratory by its resistance to bile and its fatty acid profile (21).

All of the children studied had GI symptoms severe enough to warrant diagnostic colonoscopy as part of their clinical care, and their guardians had consented to undergo research biopsies of the ileal and colonic mucosa. In some ASD patients, *Sutterella* sequences represented ~1 to 7% of the total bacterial sequences. Thus, the autism phenotype appears to have a very large effect size on the ileal mucosal composition, rivaling or even exceeding the effect size of the ileal Crohn's disease phenotype.

This study is very important for its use of mucoepithelial biopsy specimens of children with ASD and GI dysfunction. Such specimens are not easily available, and the information that they can provide about the pathophysiology of GI symptoms in ASD is likely to be of great significance. In addition, the use of a pediatric population with GI dysfunction but no autism as the control group provides an important new comparison of the microbiome in ASD versus non-ASD subjects. Finally, the pan-microbial pyrosequencing technologies that were used to separate the various *Sutterella* species are very robust.

The results of this study provide a strong rationale to conduct additional investigations of the microbiome in larger cohorts of patients with ASD and GI symptoms compared to control GI groups, as well as patients with ASD without GI manifestations and normally developing children with no GI disturbances. For the latter group, for whom intestinal biopsies are not indicated, fecal samples could be examined. Furthermore, efforts should be made to correlate the relative frequencies of *Sutterella* in ileal and fecal samples in both ASD and normally developing subjects with GI symptoms severe enough to warrant colonoscopy as part of the diagnostic workup.

REFERENCES

1. **American Psychiatric Association.** 1994. Diagnostic and statistical manual of mental disorders, 4th ed. American Psychiatric Association, Washington, DC.
2. **Ozonoff S, et al.** 2010. A prospective study of the emergence of early behavioral signs of autism. J. Am. Acad. Child Adolesc. Psychiatry **49**: 256–266.
3. **Seltzer MM, Shattuck P, Abbeduto L, Greenberg JS.** 2004. Trajectory of development in adolescents and adults with autism. Ment. Retard. Dev. Disabil. Res. Rev. **10**:234–247.
4. **Centers for Disease Control and Prevention.** 2009. Prevalence of autism spectrum disorders—autism and developmental disabilities monitoring network, United States, 2006. MMWR Surveill. Summ. 58:1–20.
5. **Sebat J, et al.** 2007. Strong association of *de novo* copy number mutations with autism. Science **316**:445–449.
6. **Miles JH.** 2011. Autism spectrum disorders—a genetics review. Genet. Med. **13**:278–294.
7. **Hornig M, et al.** 2008. Lack of association between measles virus vaccine and autism with enteropathy: a case-control study. PLoS One **3**:e3140.
8. **Institute of Medicine Safety Review Committee.** 2001. Immunization safety review: measles-mumps-rubella vaccine and autism, p 18–46. *In* Stratton K, Gable A, Shetty P, McCormick M (ed), The compass series. National Academies Press, Washington, DC.
9. **Institute of Medicine.** 2004. Immunization safety review: vaccines and autism. National Academies Press, Washington, DC.
10. **Johnson CP, Myers SM.** 2007. Identification and evaluation of children with autism spectrum disorders. Pediatrics **120**:1183–1215.
11. **Schaefer GB, Mendelsohn NJ.** 2008. Clinical genetics evaluation in identifying the etiology of autism spectrum disorders. Genet. Med. **10**: 301–305.
12. **Buie T, et al.** 2010. Evaluation, diagnosis, and treatment of gastrointestinal disorders in individuals with ASDs: a consensus report. Pediatrics **125**(Suppl. 1):S1–S18.
13. **Williams BL, Hornig H, Parekh T, Lipkin WI.** 2012. Application of novel

PCR-based methods for detection, quantitation, and phylogenetic characterization of *Sutterella* species in intestinal biopsy samples from children with autism and gastrointestinal disturbances. mBio **3**(1):e00261-11.
14. **Arumugam M, et al.** 2011. Enterotypes of the human gut microbiome. Nature **473**:174–180.
15. **Frank DN, Zhu W, Sartor RB, Li E.** 2011. Investigating the biological and clinical significance of human dysbioses. Trends Microbiol **19**:427–434.
16. **Finegold SM, et al.** 2002. Gastrointestinal microflora studies in late-onset autism. Clin. Infect. Dis. **35**:S6–S16.
17. **Parracho HM, Bingham MO, Gibson GR, McCartney AL.** 2005. Differences between the gut microflora of children with autistic spectrum disorders and that of healthy children. J. Med. Microbiol. **54**:987–991.
18. **Finegold SM, et al.** 2010. Pyrosequencing study of fecal microflora of autistic and control children. Anaerobe **16**:444–453.
19. **Adams JB, Johansen LJ, Powell LD, Quig D, Rubin RA.** 2011. Gastrointestinal flora and gastrointestinal status in children with autism—comparisons to typical children and correlation with autism severity. BMC Gastroenterol. **11**:22.
20. **Williams BL, et al.** 2011. Impaired carbohydrate digestion and transport and mucosal dysbiosis in the intestines of children with autism and gastrointestinal disturbances. PLoS One **6**:e24585.
21. **Wexler HM, et al.** 1996. *Sutterella wadsworthensis* gen. nov., sp. nov., bile-resistant microaerophilic *Campylobacter gracilis*-like clinical isolates. Int. J. Syst. Bacteriol. **46**:252–258.