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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

ORIGINAL RESEARCH

Distinct Microbiome-Neuroimmune Signatures Correlate With Functional Abdominal Pain in Children With Autism Spectrum Disorder

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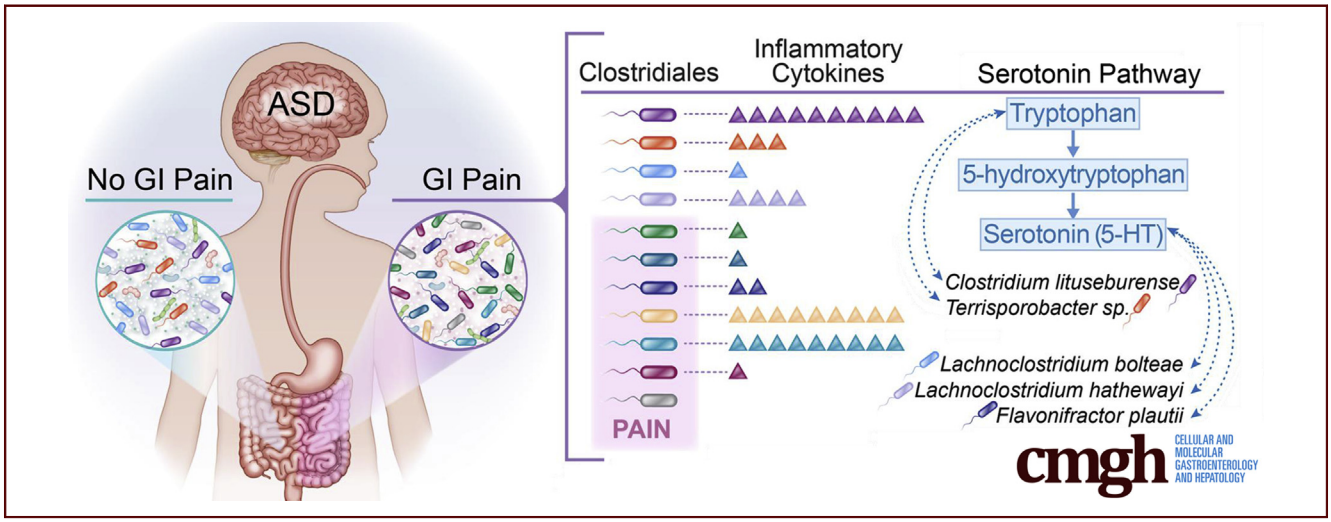

SUMMARY

We evaluated whether microbiome-neuroimmune profiles differed in children with autism spectrum disorder and functional gastrointestinal disorders, as compared with neurotypical children. Our findings identified distinctive mucosal microbial signatures in children with autism spectrum disorder and functional gastrointestinal disorders that correlated with cytokine and tryptophan homeostasis.

BACKGROUND & AIMS: Emerging data on the gut microbiome in autism spectrum disorder (ASD) suggest that altered host–microbe interactions may contribute to disease symptoms. Although gut microbial communities in children with ASD are reported to differ from individuals with neurotypical development, it is not known whether these bacteria induce pathogenic neuroimmune signals.

METHODS: Because commensal clostridia interactions with the intestinal mucosa can regulate disease-associated cytokine and serotonergic pathways in animal models, we evaluated whether microbiome-neuroimmune profiles (from rectal biopsy specimens and blood) differed in ASD children with functional gastrointestinal disorders (ASD-FGID, n = 14) compared with neurotypical (NT) children with FGID (NT-FGID, n = 15) and without abdominal pain (NT, n = 6). Microbial 16S ribosomal DNA community signatures, cytokines, and serotonergic metabolites were quantified and correlated with gastrointestinal symptoms.

RESULTS: A significant increase in several mucosa-associated Clostridiales was observed in ASD-FGID, whereas marked decreases in *Dorea* and *Blautia*, as well as *Sutterella*, were evident. Stratification by abdominal pain showed multiple organisms in ASD-FGID that correlated significantly with cytokines (interleukin [IL]6, IL1, IL17A, and interferon-γ). Group comparisons showed that IL6 and tryptophan release by mucosal biopsy specimens was highest in ASD children with abdominal pain, whereas serotonergic metabolites generally were increased in children with FGIDs. Furthermore, proinflammatory cytokines correlated significantly with several Clostridiales previously reported to associate with ASD, as did tryptophan and serotonin.

CONCLUSIONS: Our findings identify distinctive mucosal microbial signatures in ASD children with FGID that correlate with cytokine and tryptophan homeostasis. Future studies are needed to establish whether these disease-associated Clostridiales species confer early pathogenic signals in children with ASD and FGID. *(Cell Mol Gastroenterol Hepatol 2017;3:218–230; http://dx.doi.org/10.1016/j.jcmgh.2016.11.008)*

Keywords: Microbiome; Microbiome–Gut–Brain Axis; Gastrointestinal Disorders; Mucosa; Serotonin.

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Gastrointestinal (GI) dysfunction is a critical factor influencing the quality of life in individuals with autism spectrum disorder (ASD) and may contribute to behavioral challenges.[1] A clinical meta-analysis of functional GI disorders (FGID) supports the notion that GI dysfunction occurs more frequently in children with ASD than in children with neurotypical development.[2] GI disturbances ranging from constipation to diarrhea are widely documented in this population and are complicated further by the nonverbal and/or limited communication abilities of these children.[1–5]

Most occurrences of GI dysfunction in children with ASD are the result of functional causes, rather than being of anatomic or known physiologic origin.[1,3,6] Such FGIDs encompass a broad range of disorders including functional constipation, nonretentive fecal incontinence, functional abdominal pain, abdominal migraines, and irritable bowel syndrome.[7] The etiology of FGIDs is poorly understood in children with neurotypical development, let alone in ASD. A common cause of FGIDs is thought to emanate from disturbances to normal communications between the brain and gut—referred to as the *brain-gut axis*,[8] and more recently as the *microbiome–gut–brain axis*.[9,10] Altered microbiome–gut–brain signals also have been reported in ASD, and may contribute independently toward clinical symptoms by changing microbiome composition, tryptophan–serotonin imbalance, and immunologic pathways.[11–21] These preliminary reports have indicated that altered gut–brain communications not only may play a role in the increased occurrence of FGIDs in ASD individuals, but could advance our understanding of potential risk factors for FGID in the ASD community.

Differences in the types and composition of various bacterial species in children with ASD have been reported.[22–27] Most studies have focused on stool specimens because it is difficult to obtain adequate numbers of GI mucosal biopsy specimens from children with ASD. This is a potentially important deficiency in the field because mucosa-associated microbes preferentially regulate host homeostasis, as is evident in the induction of T-cell immune responses, as well as maintaining serotonin biosynthesis from dietary tryptophan in the mucosa.[28–33] Because it is unethical to perform an endoscopy on children with ASD without a clinical indication, the only cohort that can be captured is generally ASD with FGID. For this reason, it is important to compare mucosal (isolated from tissue specimens) microbiome communities in children with and without ASD and FGID because stool specimens, although providing a more feasible approach to power microbiome studies, may provide misleading insights into disease regulatory circuits in ASD. A single study, with a follow-up report, remains the only characterization of the mucosal microbiome in pediatric ASD.[23,34] Although focusing mainly on phylum level changes, the key differences associated with the ASD group with FGID were seen as an increase in Clostridiales, particularly in *Lachnospiraceae* and *Ruminococcaceae*. These preliminary reports merit deeper investigation because the Clostridiales are emerging as major microbial regulators of gut-derived T-cell immune and serotonergic signals that may be associated with ASD.[30–33,35]

Our study compared mucosa-associated microbial communities in children with ASD with previous reports characterizing stool in this population. Furthermore, we investigated whether mucosa-associated microbes correlated with altered tryptophan–serotonin metabolism and cytokine networks in clinically distinct patient cohorts. Here, we report a unique mucosa-associated microbiome signature in children with ASD that correlates significantly with quantitative cytokine and tryptophan measurements, as well as clinical symptoms. This analysis shows functional associations that distinguish both the clinical group and GI symptoms. Because mucosal-associated microbes remain poorly defined in ASD, we advance this field by linking both previously reported and new gut-microbe interactions as possible drivers of disease-associated signaling networks in ASD and in functional abdominal pain.

Materials and Methods

Study Protocol

Study participants were recruited from the outpatient pediatric GI procedure suite at Nationwide Children's Hospital in Columbus, Ohio. Participants were identified by chart reviews of males and females aged 3–18 years who were undergoing a lower endoscopy for one of the following symptoms: abdominal pain, altered stool patterns, or painless bright red blood per rectum. The research protocol was approved by the Nationwide Children's Institutional Review Board, and written consent was obtained from parents of the participants.

On the day of the procedure, participants were approached for recruitment if they met the following

Abbreviations used in this paper: **ASD, autism spectrum disorder; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, serotonin; FGID, functional gastrointestinal disorder; GI, gastrointestinal; GM-CSF, granulocyte-macrophage colony-stimulating factor; GROα, growth-related oncogene alpha; IBS, irritable bowel syndrome; IFN, interferon; IL, interleukin; IP, interferon gamma-induced protein; MCP-1, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; NT, neurotypical; OTU, operational taxonomic unit; QPGS-RIII, Questionnaire on Pediatric Gastrointestinal Symptoms-Rome III; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.**

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2352-345X
http://dx.doi.org/10.1016/j.jcmgh.2016.11.008