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EDITORIAL

Does the Human Gut Microbiota Contribute to the Etiology of Autism Spectrum Disorders?

Petra Louis

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The human gut harbors a complex microbial community (microbiota) which contributes to the breakdown of dietary constituents that are non-digestible in the upper gut. It has been recognized for some time that the activities of the microbiota exert numerous effects on the host, including the supply of energy from dietary sources otherwise unavailable to the host, modulation of host cell regulatory circuits with consequent effects on cancer risk and inflammatory tone, modulation of the host's immune system, and protection against pathogens [1, 2]. More recent studies are beginning to reveal that the actions of the microbiota have much wider effects on host physiology than originally thought, and evidence is emerging that these may include modulation of brain activity and behavior [3].

Autism spectrum disorder (ASD) encompasses neurodevelopmental disorders that are defined by behavioral observations, in particular dysfunctions in social interaction and communication skills, as well as repetitive behaviors. Several studies have revealed immune and neuronal dysregulation in autistic subjects [4, 5]. The underlying causes remain unclear, but both genetic and environmental factors appear to play a role [5–7]. The rapid increase in autism in recent years is unlikely to be explained by an increase in awareness and diagnosis of the condition alone, which also points to the environment as a contributing factor [7]. Environmental factors that may contribute to the etiology of ASD include pre- or postnatal exposure to certain environmental chemicals and drugs, stress, maternal infection, and dietary factors [7]. It has to be kept in mind that ASD envelops a range of disorders with great heterogeneity between subjects, and it may be necessary to investigate specific subgroups to identify the biological processes underlying these diseases. A subset of patients experience gastrointestinal (GI) symptoms, pointing to a role of the gut microbiota in ASD [6]. Changes in microbiota composition in subjects with ASD compared to healthy control subjects have been identified in several studies, both based on bacterial culture and molecular methods (Table 1); however, no clear trend is emerging as yet. For example, bacteroidetes have been found both to be higher and lower in ASD subjects in different studies (Table 1). Furthermore, changes in overall bacterial diversity have been reported [8], but this has not been confirmed by others [9]. A direct comparison between studies is complicated by the fact that different methodologies have been employed and that the study groups may not be directly comparable due to the heterogeneous nature of ASD (for example, ASD subjects with or without GI symptoms). Interestingly, non-autistic siblings of ASD subjects have often been found to have microbiota profiles intermediate between ASD and controls [8, 10, 11], possibly due to genetic factors, but also possibly a reflection of shared environmental conditions. These studies are further complicated by the fact that autistic subjects often receive frequent medication with antibiotics and are often on special diets or have repetitive dietary behaviors, both of which may alter microbiota composition. Thus, it is difficult to establish whether the changes seen play a causative role or are merely a consequence of the disease. A role of the microbiota in ASD is, however, supported by the fact that interventions with antibiotics have led to an improvement of behavior and communication in ASD subjects, and probiotic interventions have resulted in

P. Louis (✉)
Rowett Institute of Nutrition and Health, University of Aberdeen, Greenburn Road, Bucksburn, Aberdeen AB21 9SB, UK
e-mail: p.louis@abdn.ac.uk

Table 1 Microbiota differences determined in autism spectrum disorder subjects compared to control subjects

Study group	Methodology	Significantly higher in ASD	Significantly lower in ASD	References
13 ASD, 8 CON	Bacterial culture, feces	*Clostridium* and *Ruminococcus* spp.		Finegold et al. [16]
15 ASD, 8 CON	Quantitative PCR, feces	*Clostridium* clusters I and XI, *Clostridium bolteae*		Song et al. [17]
58 ASD, 12 SIB,10 CON	Fluorescent in situ hybridization, feces	*Clostridium histolyticum* group (*Clostridium* clusters I and II)		Parracho et al. [10]
33 ASD, 7 SIB, 8 CON	Pyrosequencing, feces	Severe ASD (11 subjects) versus CON:	Severe ASD (11 subjects) versus CON:	Finegold et al. [8]
		Phylum level: bacteroidetes and proteobacteria	Phylum level: firmicutes and actinobacteria	
		Genus level: *Alkaliflexus, Desulfovibrio, Acetanaerobacterium, Parabacteroides, Bacteroides*	Genus level: 14 genera, most significant and abundant: *Weissella, Turicibacter, Clostridium, Anaerofilum, Pseudoramibacter, Ruminococcus, Streptococcus*	
23 ASD, 22 SIB, 9 CON	Quantitative PCR, feces	*Bacteroides fragilis* in ASD subjects with GI symptoms only (9 of 23)	*A. muciniphila* (ASD and SIB); *Bifidobacterium* spp. (ASD only)	Wang et al. [11]
58 ASD, 39 CON	Bacterial culture, feces	*Lactobacillus* spp.; *Bacillus* spp.	*Bifidobacterium* spp., *Enterococcus* spp., *Klebsiella oxytoca*	Adams et al. [14]
15 ASD with GI symptoms, 7 CON with GI symptoms	Pyrosequencing and quantitative PCR, ileal and cecal biopsies	Cumulative level of firmicutes + proteobacteria; *Sutterella* spp.	bacteroidetes	Williams et al. [9, 18]

ASD Subjects with autism spectrum disorder, *SIB* non-autistic siblings, *CON* unrelated control subjects, *GI* gastrointestinal

positive effects on neuropsychological symptoms (such as mood and anxiety) in other study populations [12].

In the current issue of *Digestive Diseases and Sciences*, Wang et al. [13] report significantly higher fecal bacteria fermentation product and ammonia concentrations in 23 children with ASD compared to controls (22 non-autistic siblings and nine unrelated subjects). Total short-chain fatty acid (SCFA) levels as well as all individual SCFA, with the exception of caproic acid, and branched-chain fatty acids, were elevated in ASD subjects, pointing towards a general increase in microbiota population or activity rather than a major shift in composition, which would more likely lead to more specific changes in fermentation product profiles. The microbiota composition in the same study group had been determined previously [11], but the only significant differences found were a relative decrease in *Akkermansia muciniphila* and *Bifidobacterium* spp., with no changes in absolute levels of total bacteria. Also in this latest study [13], fecal levels of phenol and *p*-cresol were determined as markers of amino acid metabolism; however, no differences were found between the study groups. Furthermore, there was no difference in bowel movement frequency, fecal output, or fecal pH between the ASD and control group, and the significant differences remained for most fermentation products after the exclusion of subjects on special diets, antibiotics, or probiotics and of those reporting GI symptoms. In addition, no significant correlation was found between fecal fermentation product concentrations and dietary macronutrient intake as determined by questionnaire. Thus, the changes seen appeared mostly to be independent of these potential confounders. However, in absolute terms, the differences in fermentation products and ammonia between the ASD and control group, while statistically significant, were very small, with most subjects falling within the same range, putting a question mark on whether these changes are likely to be of biological consequence for the host. Furthermore, in contrast to these results, Adams et al. [14] reported lower levels of several SCFA in autistic subjects. Thus, further studies are required to establish whether bacterial fermentation products are indeed different in ASD subjects.

If changes in the gut microbiota do indeed play a role in the development of ASD, what are the likely mechanisms linking such changes to neurological development, immune function, and behavior? The SCFA propionate and butyrate have been shown to elicit behavioral changes in rodents, with propionate inducing changes similar to autism.

Whether systemic SCFA concentrations change sufficiently in response to microbial production in the colon to elicit these responses remains to be established. Furthermore, several other microbial metabolites may interact with the nervous system of the host [3]. Differences in the microbiota may also result in altered microbial metabolism of aromatic amino acids, with consequent changes in systemic metabolites (as reflected in urinary metabolite profiles), which could lead to neurological symptoms [15]. For example, *p*-cresol may affect the metabolism of dopamine [15]; however, there is no consensus on fecal and urinary differences in *p*-cresol in ASD subjects to date [13]. The microbiota could also be involved in disease etiology via interactions with the immune system [12]. Some of the possible mechanisms outlined above are more likely to involve changes within the overall balance of the whole microbial community, while others may be exerted by specific bacteria.

Further studies are required to clarify whether the gut microbiota does indeed play a role in ASD. These would ideally include prospective studies to address the question of cause and consequence, and intervention studies aimed at modulating the microbiota—for example, with probiotics or dietary interventions. Appropriate profiling and categorization of patients and control groups together with the application of state-of-the-art molecular methods to profile the microbiota and the fecal, urinary, and plasma metabolome will help to reveal the underlying mechanisms of the cross-talk between gut microbes and the host.

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