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Intestinal microbiota, metabolome and gender dimorphism in autism spectrum disorders

Rafail I. Kushak*, Harland S. Winter[1]

Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

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ABSTRACT

There is a male predominance in autism, with a male/female ratio of 4:1 and an even higher ratio (11:1) in individuals with high functioning autism. The reasons for gender differences in ASD are unknown. Genetic and environmental factors have been implicated, but no definitive evidence exists to explain male predominance. In this review, evidence is presented to support a hypothesis that the intestinal microbiota and metabolome play a role in gender dimorphism in children with autism. Metabolic products may affect not only gastrointestinal (GI) tract and the central nervous system, but also behavior, supporting communication between GI tract and central nervous system. Furthermore, mood and anxiety may affect intestinal function, indicating bidirectional flow in the gut-brain axis. Several hormone-based hypotheses are discussed to explain the prevalence of autism in males. Observations in animal models and studies in humans on the intestinal microbiome and metabolome are reviewed to support the proposed gender dimorphism hypothesis. We hypothesize that the intestinal microbiome is a contributing factor to the prevalence of ASD in boys either directly, through microbial metabolites and/or epigenetic factors capable of regulating host gene expression through DNA methylation and/or histone modification.

1. Introduction

Autism is a neurodevelopmental disorder, but GI problems including constipation, diarrhea and abdominal pain are common. Recent data from the CDC demonstrated that the frequency of autism spectrum disorders (ASD) in American boys is 1:42, but only 1:189 in girls (Home, 2014), with a male/female ratio of 4:1 (Fombonne, 2003). In high-functioning autism, the male/female ratio is even higher, at 11:1 (Gillberg, Cederlund, Lamberg, & Zeijlon, 2006). In a subgroup of individuals diagnosed with ASD without any physical or brain abnormalities as measured by MRI, the ratio was as high as 23:1 (Miles & Hillman, 2000).

The reason for gender differences in ASD is unknown. This observation is well recognized, but the reasons are poorly understood (Schaafsma & Pfaff, 2014). Investigators have sought a genetic explanation for the male prevalence in ASD, since up to 2.5% of genes in the brains of men and women are differentially expressed or spliced (Trabzuni et al., 2013). Although many genes are implicated in ASD, there are also many individuals who have alterations in those same genes and are not symptomatic (Weiss et al., 2008). Furthermore, the majority of genes implicated in autism are not located on the sex chromosomes. However, it remains possible that genes on the Y chromosome interact with ASD susceptibility genes to contribute to autism in males.

Recently, environmental factors or their interaction with human genetics were considered to play important roles in ASD etiology,

* Corresponding author at: Division of Pediatric Gastroenterology, 175 Cambridge Street, CRPZ 5, Boston, MA 02114, USA.
E-mail addresses: rkushak@mgh.harvard.edu (R.I. Kushak), hwinter@mgh.harvard.edu (H.S. Winter).
[1] Division of Pediatric Gastroenterology, 175 Cambridge Street, CRPZ 5-560, Boston, MA 02114, USA.

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especially in subjects with vulnerable phenotypes. Factors such as early exposure to androgenic hormones and early maternal immune activation could affect gender-specific susceptibility to ASD (Schaafsma & Pfaff, 2014; Pfaff, Rapin, & Goldman, 2011; Schaafsma et al., 2017).

In this review, a hypothesis involving role of the intestinal microbiota on gender dimorphism in individuals with autism is considered. The impact of three factors: (i) the intestinal microbiome; (ii) the intestinal metabolome; and (iii) gut-brain interactions in model animals and in humans contribute to the gender dimorphism hypothesis.

2. Environment and intestinal microbiota in ASD

Specific prenatal and perinatal environmental factors are implicated to increase ASD risk (Gardener, Spiegelman, & Buka, 2011), to the extent that some investigators suggest that in ASD environmental factors may be more important than genetic factors (Hallmayer, Cleveland, & Torres, 2011). Environmental factors include not only external factors such as xenobiotics, hormones, inflammatory agents and early stress, but also host environmental factors such as intestinal microbiota. There are more than 1000 microbial species living in the human intestine and dysbiosis of the intestinal microbiota may be associated with various diseases (metabolic diseases, intestinal disorders, cancer, etc.) including autism. Malabsorption in patients with autism might be related to a disruption of the indigenous microbiota promoting the overgrowth of potentially pathogenic microorganisms, such as *Clostridia.* Some of these bacteria are known to produce toxins, including neurotoxins, lethal toxins, oxygen-liable hemolysins, binary toxins and ADP-ribosyltransferases (Hatheway, 1990). Using traditional cultivation methods that provide information on a limited number of bacterial species inhabiting the gastrointestinal tract, Finegold et al. (2002) demonstrated nine species of *Clostridium* in the stool of children with autism that were not found in stool from healthy subjects. Implementation of modern molecular methods including next generation sequencing techniques allowed more extensive evaluation of the microbiome. Subsequent work by Finegold's group using real-time PCR quantified some *Clostridium* clusters and the species *Clostridium bolteae* in the stool of children with ASD (Song, Liu, & Finegold, 2004). The higher incidence of *Clostridium*, specifically *C. histolyticum*, in children with autism as compared with a healthy control group was subsequently confirmed by Parracho et al. (Parracho, Bingham, Gibson, & McCartney, 2005) using fluorescence *in situ* hybridization with 16S rRNA-based oligonucleotide probes. They suggested a possible link between clostridial levels and GI function in ASD patients.

In other studies investigators demonstrated significant microbial dysbiosis in the stool of children with autism as compared with that of unaffected children. Finegold et al. (Finegold, Dowd, & Gontcharova, 2010), using bacterial amplicon pyrosequencing technology, reported decreased *Firmicutes* and *Actinobacteria* and increased *Bacteriodetes* and *Proteobacteria* in the stool of children with autism when compared to controls. In addition, *Desulfovibrio* species and *Bacteroides vulgatus* were present in significantly higher numbers in stools of children with severe autism when compared with controls. The stool of children with autism also contained lower numbers of *Bifidobacterium* species and the mucolytic bacterium *Akkermansia muciniphila* (Wang et al., 2011). Kang et al. (2013) demonstrated a less diverse microbiome in the stool of individuals with autism than in unaffected controls, with lower levels *of Prevotella, Coprococcus*, and unclassified Veillonellaceae. They noted that the simplified microbiome in the study population was associated with the presence of symptoms of autism rather than the severity of GI symptoms. A less diverse gut bacterial population in children with ASD compared with neurotypical controls was confirmed in a recent study by the same group (Kang et al., 2017). Changes in microbiome diversity were found also by De Angelis, Piccolo, and Vannini (2013), who analyzed the intestinal microbiome and metabolome in children with autism and pervasive developmental disorder not otherwise specified (PDD-NOS) in comparison with neurotypical controls. They found more changes in the intestinal microbiota and metabolome in PDD-NOS individuals, and especially children with autism, than in healthy controls, and hypothesize that the degree of microbial alteration correlates with the severity of the disease. Other investigators reported that the stools of children with autism contain lower levels of beneficial bacteria such as *Bifidobacterium*, slightly lower levels of *Enterococcus*, and much higher levels of *Lactobacillus* in comparison with unaffected children (Adams, Johansen, Powell, Quig, & Rubin, 2011). More detailed information on changes in the stool microbiota of children with autism in comparison with controls can be found in recent reviews (De Angelis, Francavilla, Piccolo, De Giacomo, & Gobbetti, 2015; Krajmalnik-Brown, Lozupone, Kang, & Adams, 2015; Ding, Taur, & Walkup, 2017; Li, Han, Dy, & Hagerman, 2017).

Compositional dysbiosis was found also in the ileum of children with autism. Metagenomic analysis demonstrated a decrease in *Bacteroidetes*, increased ratio of *Firmicutes* to *Bacteroidetes* and an increase in Betaproteobacteria (Williams et al., 2011). In another study, the same authors reported that *Sutterella* represented a major component of the ileal mucosal microbiota in over half of the children with autism along with gastrointestinal dysfunction, but was absent in neurotypical children with GI disorders (Williams, Hornig, Parekh, & Lipkin, 2012). Bacterial dysbiosis or an overgrowth of a specific population of bacteria was found not only in the colon and ileum, where microbiota represented mostly by Gram-negative species, but also in the duodenum, which is populated predominantly by Gram-positive microorganisms from the oropharynx (Williams et al., 2012; Riordan et al., 2001; Simon & Gorbach, 1986). A significant number of non-spore-forming anaerobes and microaerophilic bacteria were identified in the duodenal fluid of children with autism in contrast to the total absence of such bacteria in control children (Finegold et al., 2002). The mucosal adherent bacterial in the duodenal mucosa of children with autism demonstrated different populations of bacteria. Bacteria belonging to the genus *Burkholderia* were more abundant in subjects with autism, while members of the genus *Neisseria* were higher in unaffected controls. At the species level, a relative decrease in abundance of two *Bacteroides* species and *Escherichia coli* was found in individuals with autism. Interestingly, in individuals with autism, disaccharidase activity correlated with the abundance of *Clostridium* species (Kushak et al., 2016).

Thus, data from multiple studies show that an abnormal gut microbiota is related to ASD. Microbial dysbiosis in children with autism was found not only in the stool and colonic mucosa, but also in mucosa of the small intestine. Observations about the