Linking Gut Dysbiosis and Neuroinflammation Observed in Individuals with Autism Spectrum Disorder and Possible Treatment with Bacteroides Fragilis

Abstract
Autism spectrum disorder (ASD) is a term that encompasses neurodevelopmental disorders characterized by behavioural, communication, and social deficits. In addition to this triad of impairments, changes related to cellular processes including gut permeability, neuro anatomical alterations and neuro-inflammation have also been identified in individuals with ASD. Patients with ASD also demonstrate altered proportions of bacteria in the gut. Since the gut microbiome has a significant impact on many physiological processes including those beyond the gut, dysbiosis observed in ASD has been the subject of recent research investigating its implications in the aforementioned pathways. This review will explore the specific cellular processes drawn from this research. The specific dysbiosis observed in ASD will be defined and linkages will be drawn to relate bacterial processes to the aberrant cell processes in ASD. Microbiota-related treatments will also be outlined based on the current understanding of the dysbiosis-ASD relationship. Finally, the evidence reviewed here will outline areas of ambiguity and provide a foundation for further research into specific pathways.

Keywords: Autism spectrum disorder; Gut permeability; dysbiosis; Neuro-inflammation

Introduction
Autism Spectrum Disorder (ASD) is a range of neurodevelopmental disorders that demonstrate the triad of impairments: difficulties in social and emotional understanding, difficulties in communication, and repetitive, stereotyped behaviours and/or interests [1]. As a diagnostic term, ASD has in the fifth edition of the Diagnostic and Statistical Manual (DSM5) replaced what DSM-IV classified as Pervasive Developmental Disorders (PDDs), a group of disorders that encompassed five defined disorders: Asperger’s disorder, Autistic disorder, Rett’s Disorder, Childhood Degenerative Disorder, and Pervasive Developmental Disorder Not Otherwise Specified [1]. Each of the PDDs display different behavioural, motor, or intellectual abnormalities but are nonetheless centered around the triad of impairments. Current understanding attributes ASD to the influence of both genetic and environmental factors, where genetic predisposition is highly affected by external variables, such as epigenetics [2].

ASD manifests itself as a wide range of symptoms. Patients with ASD not only display the classic triad of impairments, but may also present psychological abnormalities such as attention deficits, sensory hyper- and/or hypo-sensitivities, and behaviours that may be aggressive or self-harming in nature [3,4]. Patients with ASD may have a higher prevalence of seizures, insomnia, and ear infections [3]. Notably, an increased prevalence of GI symptoms such as constipation, diarrhoea, bloating, abdominal pain, reflux, vomiting, and flatulence are also characteristic of ASD [5].

The pathophysiological factors that have been associated with ASD symptoms include structural brain differences, chronic neuro-inflammation, oxidative stress, mitochondrial dysfunction, and gut permeability [6-13]. Many studies have demonstrated that brain regions associated with functions that are impaired in ASD have altered activation, reduced or altered connectivity, and distinct integration between different brain areas [14,15]. Additionally, several biomarkers and endo-phenotypes have been associated with ASD such as biomarkers for oxidative damage, blood cytokine levels, and metabolites of mitochondrial functions [16,17]. However, it is important to note that the external validity of many studies is limited by small sample sizes, reproducibility challenges and the heterogeneity of genetic pathways to ASD.

Studies show that over 70% of ASD patients currently experience or have a history of gastrointestinal (GI) complications, suggesting a link between ASD and gut abnormalities [18]. Patients with ASD often have altered microbial compositions, such as increased Clostridium (p=0.0393), Desulfovibrion (p=0.011), and Bacteroides...
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Fluid where they then directly interact with cells of the central nervous system (CNS) activity, raising the hypothetical possibility of neuro developmental pathogenesis through alterations in normal brain-gut signalling [22]. The pathogenic microbiota may increase immune-mediated inflammation, compete for binding sites on enteric walls with commensal bacteria, or regulate pathways via production of metabolites [23]. One of the main mechanisms that we will examine is how bacterial products may enter systemic circulation and pass through the blood-brain barrier to alter neurobiology. Specifically, we will be discussing how heightened gut permeability and neuro immune dysregulation may be implicated in the etiology of ASD, as well as the viability of probiotic treatment.

Gut Permeability

ASD is comorbid with many gastrointestinal complications involving increased gut permeability [24-27]. The physical integrity of the luminal barrier is often assessed through the sugar permeability test, which involves simultaneous oral delivery of the saccharides lactulose and mannitol, followed by measuring the urinary lactulose:mannitol ratio [28,29]. Unlike mannitol, lactulose is too bulky to traverse the luminal mucosa via aqueous pores of the intestinal epithelium [30,31]. For serum lactulose molecules to arise, lactulose must travel in between epithelial cells at areas of cell extrusion [30,32]. Through the sugar permeability test, D’Eufemia et al. [24] reported a significantly elevated urinary lactulose:mannitol ratio in 43% of patients with ASD [24]. Increased serum lactulose suggests the presence of leaky tight junctions linking together adjacent intestinal epithelial cells [24,32,33]. This destabilization of the luminal epithelium allows entry of certain food antigens that induce ASD-related antigenic responses [34] and neurological dysfunction [35]. The propagation of the immune response further damages the mucosa through zonulin-mediated positive feedback [36,37].

Zonulin modulates intercellular tight junctions involved in transportation of macromolecules from the lumen into intestinal tissue [36-38]. It is therefore necessary to the regulation of immune responses and tolerance [36,39]. Both intestinal and extra-intestinal inflammatory disorders can arise when the zonulin pathway is deregulated in genetically susceptible individuals [36,40].

Food-derived opioid-like peptides can be referred to as exorphins due to their structural and functional similarities with endorphin, a type of opioid hormone [41,42]. Prolonged opiate exposure in animal models is shown to yield behavioural symptoms comparable to those in children with ASD, namely, repetitive actions and social disengagement [43]. Noting the implications of diet on schizophrenia, studies speculated that a faulty intestinal barrier allows neuro active food derivatives or exorphins to travel into the blood and later the cerebrospinal fluid where they then directly interact with cells of the central nervous system [44,45]. Although there is conflicting evidence concerning whether endogenous endorphins are elevated in autism. In addition to entry of incompletely digested foods, increased gut permeability has also allowed the increased levels of serum pathogenic compounds arising from gut dysbiosis [46,47]. Specifically, we will be examining propionic acid, a short-chain fatty acid produced during bacterial fermentation, as well as the release of lipopolysaccharides from the membrane of gram-negative bacteria. Through blood circulation, these compounds are able to travel from the gut lumen to the CNS where they interfere with cellular respiration, microglia regulation, and other pathways that may be related to the neurobiology of ASD [48,49].

Maternal Immune Activation (MIA) Murine models and Bacteroides fragilis Treatment

Experiments on mice have shown that infections leading to maternal immune abnormalities during pregnancy can lead to symptoms equivalent to those seen in ASD, providing further evidence that some environmental factors altering maternal and fetal immune systems may lead to fetal predisposition to ASD [50,51]. Applying the findings from observational studies on human ASD, an ASD murine model was constructed through maternal immune activation, where pregnant mice were injected with immune-activating polyinosinic:polycytidylic acid (poly I:C) to produce offspring that served as phenotypic models of ASD [52-54]. Offspring of MIA mice exhibit ASD-like pathologies and symptoms, such as defects in intestinal integrity, alterations in the microbiome composition, intestinal cytokine levels and metabolome profiles analogous to that subsets in the human ASD population [54,55]. Symptomatic similarities between human ASD and MIA offspring suggest that the MIA offspring serve as reliable models of ASD. Most of ASD-associated behavioural and metabolic symptoms in these mice were ameliorated through oral treatment of Bacteroides fragilis, a bacterial species shown effective against colitis [52]. MIA-induced microbiome had mild elevations in Clostridia and Bacteroides populations [55]. In the same study researchers noted that although there was insignificant difference in the relative abundance of Clostridia (p=0.8340) and Bacteroidia (p=0.9943) between MIA offspring and controls, the microbial species that experienced slightly greater proliferation within the MIA offspring than within the controls belonged to one of the two classes. The altered microbiome in ASD model mice aligns with studies reporting elevating Clostridium levels in the fetal matter of ASD individuals [56-58]. The exception to the increase in certain Bacteroides (12.02±1.62% control, 13.48±0.75% MIA) and Clostridium (1.00±0.25% control, 1.58±0.34% MIA) species was B. fragilis, which was notably decreased in MIA offspring [52]. Supplementation of B. fragilis back into the intestinal environment of MIA offspring modified the microbiome composition such that it was comparable to that of the controls [52]. There was however no significant effect on the overall gut microbiotal abundance or diversity (microbiome density p=0.2980, species diversity p=0.5440) upon B. fragilis treatment [52].

Administration of B. fragilis also normalized colonic elevation of IL-6 in MIA offspring [59]. IL-6 is critical cytokine involved in MIA pathways linked to abnormal fetal brain development and predisposition of offspring to neuro development pathologies.

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MIA offspring also exhibited decreased prepulse inhibition at lower decibels, which is reflective of behaviour defects [52]. Poor startle response, a common trait among autistic patients, is indicative of impaired sensorimotor gating [62,63]. Behaviour assays also showed deficits in duration and frequency of vocalization, sociability, and demonstration of repetitive behaviour, which are often considered diagnostic symptoms of ASD [52]. However, B. fragilis-treated MIA offspring experienced improved behavioural, communicative and sensorimotor responses despite retaining deficits in sociability and exploratory propensity [52]. Similarly, risperidone, an antipsychotic that is used in the management of irritability and aggression in ASD, was unable to correct social disengagement in both ASD individuals and genetic murine model for ASD [64-67]. From these results, it is hypothesized that social behaviour is governed by a complex set of neuronal circuitry [52], which may not entirely overlap with pathways modulated by B. fragilis or atypical psychotropics.

Metabolomic assessments show that MIA has substantial impact on 8% of serum metabolites [59]. Relative to controls, MIA offspring are found to have a 46-fold increase in 4-ethylphenylsulphate (4EPS) and a moderate increase in indolepyruvate [52]. The striking elevation in 4EPS led to secondary experiments that showed how increasing 4EPS is sufficient in bringing about ASD-related behavioural abnormalities in previously naïve mice [52]. It is also speculated that 4EPS production is exclusively regulated by commensal microbiota since germ-free mice only display trace amounts of serum 4EPS [52]. These results demonstrate how gut microbes may lead to ASD-related behaviour through dysregulation of systemic metabolites levels. Interestingly, 4EPS and indolepyruvate also possess structural equivalents to human metabolites p-cresol and indolyl-3-acetylglucine (IAG) [52]. Although there are conflicting reports regarding whether the increase in IAG levels between ASD groups and controls is statistically significant p-cresol is postulated to be a urinary biomarker for ASD [68-71]. Not unlike other MIA-induced symptoms, atypical metabolic profiles were normalized by B. fragilis supplementation [52]. Overall, the findings suggest that probiotic supplementation can be a viable treatment for symptoms of through rectifying gut dysbiosis.

Conclusion and Perspectives

Many studies have proposed links between the etiology of ASD symptoms and gut dysbiosis. Given the high prevalence of increased gut permeability in ASD patients, it is hypothesized that poor luminal integrity is an avenue by which bacterial compounds access the central nervous system impair neuronal function. Changes in gut microbiota may result in response to oxidative stress but also further propagate it. Chronic neuroinflammation, which characterized by neuroglial activation and altered profiles of cytokines, chemokines, and growth factors, is also observed in individuals with ASD. The association between gut flora and neuro developmental disorders is further supported by a maternal immune activation study where phenotypic models of ASD displayed decreased colonies of B. fragilis. Interestingly, many ASD symptoms were normalized in MIA offspring upon recovery of B. fragilis levels through oral administration. These findings demonstrate that alterations in the gut microbiome can contribute to the development of ASD-associated symptoms.

An examination of all the evidence presented in this review shows a probable link between gut dysbiosis and ASD, however, the nature of this link should be explored in more depth. Further research is required to better establish the specific changes in individuals with ASD that are related to gut permeability and neuro-inflammation, as well as the mechanisms underlying the interplay between these changes and microbiota. The relationship between gut dysbiosis and ASD is a field where there are currently a greater number of questions than answers: there is a need for studies with larger sample sizes and better methods for testing gut dysbiosis in individuals. As treatments targeting gut dysbiosis and microbiota-associated pathways are being explored as an avenue ASD treatment, this field still provides a worthwhile basis for further exploration.

References
1. Kenneth S (2013) Neuro developmental Disorders. Diagnostic and Statistical Manual of Mental Disorders. American Psychiatric Association 5.
2. Ruggeri B, Sarkans U, Schumann G, Persico A (2014) Biomarkers in autism spectrum disorder: the old and the new. Psychopharmacol 231(6): 1201-1216.
3. Barbaresi W, Katusic S, Voigt R (2006) Autism. Archives of Pediatrics & Adolescent Medicine 160(11): 1167.
4. Mikes J (2011) Autism spectrum disorders-A genetics review. Genet Med 13(4): 278-294.
5. McElhanon B, McCracken C, Karpen S, Sharp W (2014) Gastrointestinal symptoms in autism spectrum disorder: a meta-analysis. Pediatrics 133(5): 872-883.
6. Zeidán Chulá F, Salmina A, Malinovskaya N, Noda M, Verkhratsky A, et al. (2014) The glial perspective of autism spectrum disorders. Neurosci Biobehav Rev 38: 160-172.
7. Kraneveld A, De Theije C, van Heesch F, Borre Y, de Kivit S, et al. (2014) The neuro-immune axis: prospect for novel treatments for mental disorders. Basic Clin Pharmacol Toxicol 114(1): 128-136.
8. Frustaci A, Neri M, Cesario A, Adams J, Domenici E, et al. (2012) Oxidative stress-related biomarkers in autism: Systematic review and meta-analyses. Free Radic Biol and Med 52(10): 2128-2141.
9. Rose S, Frye R, Slattery J, Wynne R, Tippett M, et al. (2014) Oxidative stress induces mitochondrial dysfunction in a subset of autism lymphoblastoid cell lines in a well-matched case control cohort. PLoS One 9(1): e85436.
10. Shpyleva S, Ivanovskiy S, De Conti A, Melnychuk S, Tryndyk V, et al. (2014) Cerebellar oxidative DNA damage and altered DNA methylation in the BTBR T+tf/J mouse model of autism and similarities with human post mortem cerebellum. PLoS One 9(11): e113712.
11. Rossignol D, Frye R (2012) Mitochondrial dysfunction in autism spectrum disorders: a systematic review and meta-analysis. Mol Psychiatry 17(3): 290-314.
12. Heberling C, Dhurjati P, Sasser M (2013) Hypothesis for a systems connectivity model of autism spectrum disorder pathogenesis.
Fadeyev AV, (2013) Gliadin, zonulin and gut permeability: effects on celiac disease and type 1 diabetes paradigms. Ann N Y Acad Sci 1165: 195-205.
30. Fasano A (2008) Physiological, pathological, and therapeutic implications of zonulin-mediated intestinal barrier modulation. Am J Pathol 1173(5): 1243-1252.
31. Visscher J, Roizing J, Sapone A, Lammers K, Fasano A (2009) Tight junctions, intestinal permeability, and autoimmunity celiac disease and type 1 diabetes paradigm. Ann N Y Acad Sci 1165: 195-205.
32. Koch G, Wiedemann K, Teschemacher H (1985) Opioid activities of human beta-casomorphins. Naunyn Schmiedebergs Arch Pharmacol 133(4): 351-354.
33. Lindstrom LH, Nyberg F, Tenerius L, Bauer K, Besve G, et al. (1984) CSF and plasma beta-casomorphin-like opioid peptides in postpartum psychosis. Am J Psychiatry 141(9): 1059-1066.
34. Parkes K, (1979) A neurochemical theory of autism. Trends in Neurosciences 2: 174-177.
35. Dohan FC (1980) Hypothesis: genes and neuroactive peptides from food as cause of schizophrenia. Adv Biochem Psychopharmacol 22: 175-192.
36. Nankova BB, Agarwal R, MacFabe DF, La Gamma EF (2014) Enteric bacterial metabolites propionic and butyric acid modulate gene expression, including CREB dependent catecholaminergic neurotransmission, in PC12 cells possible relevance to autism spectrum disorders. PLoS ONE 9(8): 103740.
37. Jyonouchi H, Sun S, Itozaki N (2002) Innate immunity associated with inflammatory responses and cytokine production against common dietary proteins in patients with autism spectrum disorder. Neuropsychobiology 46(2): 76-84.
38. Finegold SM, Molitoris D, Song Y, Liu C, Vaisanen ML, et al. (2002) Gastrointestinal microbiota and metabolite biomarkers in children with autism spectrum disorders. Biomark Med 8(3): 331-344.
39. De Magistris L, Familiari V, Pascotto A, Sapone A, Frolli A, et al. (2010) Alterations of the intestinal barrier in patients with autism spectrum disorders and in their first-degree relatives. J Pediatr Gastroenterol Nutr 51(4): 418-424.
40. Ibrahim SH, Voigt RG, Kutsic SK, Weaver AL, Barbarese WI (2009) Incidence of gastrointestinal symptoms in children with autism: a population-based study. Pediatrics 124(2): 680-686.
41. Bjarnason I, MacPherson A, Hollander D (1995) Intestinal permeability: an overview. Gastroenterology 108(5): 1566-1581.
42. Fasano A (2011) Zonulin and its regulation by zonulin: diagnostic and therapeutic implications. Clin Gastroenterol Hepatol 10(10): 1096-1100.
43. Nelson A, Wiedemann K, Teschemacher H (1985) Opioid activities of human beta-casomorphins. Naunyn Schmiedebergs Arch Pharmacol 331(4): 351-354.
44. Foster JA, McVey Neufeld K (2013) Gut-brain axis: how the microbiome influences anxiety and depression. Trends in Neurosciences 36(5): 305-312.
45. D'Eufemia P, Celli M, Finocchiaro R, Pacifico L, Viozzi L, et al. (1996) Abnormal intestinal permeability in children with autism. Acta Paediatr 85(9): 1076-1079.
46. Van De Sande M, van Buul V, Brunus F (2014) Autism and nutrition: the role of the gut-brain axis. Nutr Res Rev 27(2): 199-214.
47. Wang L, Conlon M, Christophersen C, Sorich M, Angley M (2014) Gastrointestinal microbial community and metabolite biomarkers in children with autism spectrum disorders. Biomark Med 8(3): 331-344.
48. De Theije C, Wu J, da Silva S, Kamphuis P, Garssen J, et al. (2011) Pyrosequencing study of fecal microflora of autistic and control children. Front Neurosci 5(Suppl 1): 56-516.
49. Finogold SM, Moltorits D, Song Y, Liu C, Vaisanen ML, et al. (2002) Gastrointestinal microflora studies in late-onset autism. Clin Infect Dis 35(Suppl 1): S6-S16.
50. Finogold S, Dowd S, Gontcharova V, Liu C, Henley K, et al. (2010) Pyrosequencing study of fecal microflora of autistic and control children. Anaoerobe 16(4): 443-453.
51. De Theije C, Wu J, da Silva S, Kamphuis P, Gasssen J, et al. (2011) Pathways underlying the gut-brain connection in autism spectrum disorders as future targets for disease management. Eur J Pharmacol 668(Suppl 1): S70-S80.
52. Borre Y, O’Keefe G, Clarke G, Stanton C, Dinan T, et al. (2014) Microbiota and neurodevelopmental windows: implications for brain disorders. Trends Mol Med 20(9): 509-518.
53. Foster JA, McVey Neufeld K (2013) Gut-brain axis: how the microbiome influences anxiety and depression. Trends in Neurosciences 36(5): 305-312.
54. D'Eufemia P, Celli M, Finocchiaro R, Pacifico L, Viozzi L, et al. (1996) Abnormal intestinal permeability in children with autism. Acta Paediatr 85(9): 1076-1079.
55. Boukhiri S, Matoussi N, Belhadj A, Mammou S, Diafa SB, et al. (2010) [Abnormal intestinal permeability in children with autism]. Tunis Med 88(9): 685-686.
56. De Magistris L, Familiar V, Pascotto A, Sapone A, Frolli A, et al. (2010) Alterations of the intestinal barrier in patients with autism spectrum disorders and in their first-degree relatives. J Pediatr Gastroenterol Nutr 51(4): 418-424.
57. Ibrahim SH, Voigt RG, Kutsic SK, Weaver AL, Barbarese WI (2009) Incidence of gastrointestinal symptoms in children with autism: a population-based study. Pediatrics 124(2): 680-686.
58. Bjarnason I, MacPherson A, Hollander D (1995) Intestinal permeability: an overview. Gastroenterology 108(5): 1566-1581.
59. Hodges S, Ashmore SP, Patel HR, Tanner MS (1999) Collapsible: mannitol differential permeability in small bowel disease. Arch Dis Child 64(6): 853-855.
60. Travis S, Mienzi I (1992) Intestinal permeability: functional assessment and significance. Clin Sci (Lond) 82(5): 471-488.
61. Johnston SD, Smye M, Watson RG, McMillan SA, Trimbble ER, et al. (2000) Lactulose-mannitol intestinal permeability test: a useful screening test for adult coeliac disease. Ann Clin Biochem 37(4 Pt 4): 512-519.
62. Barboza Junior MS, Silva TM, Guerrat RL, Lima AA (1999) Measurement of intestinal permeability using mannitol and lactulose in children with diarrheal diseases. Braz J Med Biol Res 32(12): 1499-1504.
63. Hamilton I, Hill A, Bose B, Bouchier IA, Forsyth JS (1987) Small intestinal permeability in pediatric clinical practice. J Pediatr Gastroenterol Nutr 6(5): 697-701.
64. Zioudrou C, Srearty RA, Klee WA (1979) Opioid peptides derived from food proteins. The exorphins. J Biol Chem 254(7): 2446-2449.
65. Paul S, Kennedy A, Rowell F, Remy T (1970) Role of nerve peptides in autism and their relationships with classical neurotransmitters. Brain Dysfunct 3(5-6): 328-345.
66. Fasano A (2011) Zonulin and its regulation by zonulin: diagnostic and therapeutic implications. Clin Gastroenterol Hepatol 10(10): 1096-1100.
67. Fasano A (2008) Physiological, pathological, and therapeutic implications of zonulin-mediated intestinal barrier modulation. Am J Pathol 1173(5): 1243-1252.
68. Visscher J, Roizing J, Sapone A, Lammers K, Fasano A (2009) Tight junctions, intestinal permeability, and autoimmunity celiac disease and type 1 diabetes paradigm. Ann NY Acad Sci 1165: 195-205.
69. Koch G, Wiedemann K, Teschemacher H (1985) Opioid activities of human beta-casomorphins. Naunyn Schmiedebergs Arch Pharmacol 133(4): 351-354.
70. Lindström LH, Nyberg F, Tenerius L, Bauer K, Besve G, et al. (1984) CSF and plasma beta-casomorphin-like opioid peptides in postpartum psychosis. Am J Psychiatry 141(9): 1059-1066.
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