Austin Journal of Autism & Related Disabilities

Open Access

Austin Publishing Group

Review Article

# Autism: "Leaky Gut", Prematurity and Lactoferrin

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**Received:** April 06, 2016; **Accepted:** May 27, 2016; **Published:** May 31, 2016

**Abstract**

Children with autism frequently suffer from a variety of gastrointestinal (GI) problems, referred to as "leaky gut", exhibit increased levels of pathogenic bacterial species, and interestingly, show higher prevalence of inflammatory bowel disease (IBD). It has been proposed that abnormal gut development may contribute to these problems. In turn, delayed gut development may affect the gut-brain axis communication and lead to altered brain development implicated in autism. In the premature infants IBD occurs primarily in the form of necrotizing enterocolitis (NEC). Importantly, the rate of both premature births and autism has been on an increase. These observations suggest that the GI problems present in subpopulations of autistic children may be related to prematurity. The preterm infant's gut with underdeveloped gut lining, affects its ability to make a transition to the external milieu and the first bacterial colonization, which affect the subsequent development of the gut and the gut-brain axis impacting brain development. Lower levels of the brain-derived neurotrophic factor (BDNF) in premature infants may further affect brain maturation. Additionally, premature infants are often fed formulas devoid of essential bioactive factors such as lactoferrin (LF), present in the human colostrum and milk, and critical for both gut and brain maturation. Even when LF is used as a supplement, in infant formulas, it is present in low concentration and is mostly of bovine origin; recent research suggests species-specificity of LF. This review discusses: (1) the delay in gut maturation in preterm infants, (2) early nutritional practices in preterm infants, and (3) species-specific structure-function relationship of LF. It considers the possible link between gut immaturity and autistic pathology. It concludes with a suggestion that breastfeeding or/and native human LF supplementation may play a preventive role in autism.

**Keywords:** Lactoferrin; Inflammatory bowel disease; Brain-derived neurotrophic factor

## Introduction

In addition to neuropsychiatric symptoms, children with autism spectrum disorders (ASD) frequently suffer from a variety of gastrointestinal (GI) problems [1]. These GI problems, in which intestinal lining/the gut-blood barrier is hyper permeable to bacteria, viruses, parasites, proteins and toxins, allowing them to be transferred between the gut and the bloodstream, are referred to by an umbrella term - the "leaky gut syndrome" [2]. The "leaky gut syndrome" underlies the pathologies of the inflammatory bowel disease (IBD), which in turn encompasses necrotizing enterocolitis (NEC), Crohn's disease, and ulcerative colitis. The "leaky gut" is also observed in a subpopulation of ASD, where it is associated with increased levels of pathogenic bacterial species in the gut [3]. Interestingly, the prevalence of GI problems, including IBD and especially NEC appears to be higher in the premature as compared to term children. Furthermore, the rate of both premature births [4] and autism [5] has been to increase. The gut-blood barrier is absent at birth in the term infants, and is established as part of the maturation of the GI system in the first few months of life [6]. In the preterm infants, there may be an additional delay in gut development, as suggested by persistent delay in maturation of digestive functions during the postnatal period in the preterm pigs [7]. Furthermore, in the term infants, once the barrier is established, a number of factors that cause inflammation of the gut lining, such as antibiotics, nonsteroidal anti-inflammatory drugs (aspirin, ibuprofen), food contaminated by parasites, highly refined carbohydrate diet (candies), gluten, enzyme deficiencies and genetically modified foods can cause "leaky gut syndrome". In preterm infants some of the same factors may have more disrupting effects than in term infants. Animal studies suggest that gut leakiness [8] or changes in the gut microbiota [9] may in turn be communicated to the brain impacting neurodevelopment. The discovery that altered gut microbiota composition can affect behavior and cognition contributed to establishing the concept of the microbiota-gut-brain axis as an extension of the concept of gut-brain axis. Thus, abnormalities in gut development, and the "leaky gut" may in fact result in brain development abnormalities implicated in autism [10]. In mammals, the developmental process, including gut maturation, is intimately dependent on bacterial colonization and early nutrition. In humans, the colostrum and milk provide not only basic nutrients, but also bioactive substances of which lactoferrin (LF) may play a key function. LF, plays an important role in the maturation of the gastro intestinal tract (GIT), early immune response and neurodevelopment. Its role in IBD has been subject of a number of studies; fewer studies concern the use of LF in autism. Preterm infants are often deprived of breastfeeding that provides native LF, while formulas are supplemented predominantly by bovine and less often by genetically modified LF. However, recently it has been reported that LF appears to be species specific, with differences in both protein moiety [11] and glycosylation pattern [12], intimately involved in both gut and brain




**Citation:** Makowska M, Kasarello K, Bialy M and Sajdel-Sulkowska EM. Autism: "Leaky Gut", Prematurity and Lactoferrin. Austin J Autism & Relat Disabil. 2016; 2(3): 1021.

maturation. Others have reported species differences in glycosylation patterns and antimicrobial activity in genetically modified LF [13]. In view of these findings, while mindful of the current progress in saving the lives of premature infants, one should pose a question whether or not one should be more cautious when designing infant formulas? This review addresses the possibility of prematurity, and specifically delayed maturation of the GI and resulting gastrointestinal problems, as the predisposing factors in autism. The immaturity of the gut at the time of preterm birth affects its ability to undergo proper bacterial colonization, impairs further gut development, and the gut-brain axis maturation, impacting brain development that may predispose autism. We further examine the potential role of breastfeeding and/or the use of native human LF in prevention of autism.

## Autism and Preterm Births: Increase in Prevalence and IBD Occurrence

The prevalence of ASD in USA has more than doubled over the last two decades [5,14,15]. The median global prevalence rate of ASD published in 2012 is over 62 cases per 10,000 people [16]. At the same time the rate of premature infants, defined as born before the 37 weeks of gestation, has increased by 20 percent between 1990 and 2006 contributing to a greater number of infants at higher risk for autistic-like behavior later in life [4]. Increasing prevalence of IBD in general population has also been observed [17-19]. Furthermore, the age-adjusted increase in the prevalence of IBD in autism, based on the case-control studies, has been recently reported [20]. Similar correlation has been claimed between IBD and prematurity. Premature infants and especially very-low-birth-weight infants experience GI dysfunctions, sepsis, and NEC that result in altered gut microbiota and dysbiosis [21]. NEC, a form of IBD, affects primarily premature infants, and is a lethal cause of infant morbidity and mortality. It has been suggested that the microbial intestinal colonization may affect the immature gut establishing inflammatory and barrier properties predisposing to the development of NEC [22]. Additional data support the notion of preterm birth as a risk factor of IBD in adulthood [23]. Others reported that there is no relationship between preterm births and development of IBD in adulthood [24]. Additional data is needed to clarify the effect of early childhood events on the development of IBD. Early life factors have been postulated to play a role in the development of the immune tolerance and microbiome, which in turn may influence the risk of IBD, although the exact relationship is unclear. Premature infants [25,26] and especially those with a very-low-birth-weight [27] are at risk of specific learning deficits, hyperactivity, impaired social interactions and autism. Furthermore, the impairment of social behavior may persist into adulthood [28]. Magnetic resonance imaging (MRI) scans in very premature babies, subsequently diagnosed with autism, showed reduced volumes in the temporal, occipital, insular and limbic region, which are involved in autistic pathology [29]. Importantly, clinical studies indicate a relationship between the premature birth and the age of first signs of autistic symptoms, which is at 7 months in preterm and 13 months in term infants [30].

## Early Nutritional Practices in Term and Preterm Infants

Management practices in preterm infants, as well as in term infants during infection, frequently employ interruption in nursing. Such practices may contribute to infant's deficiency in bioactive factors such as LF, as well as several hormones including critical for the development thyroid hormone (TH), and have dire consequences on both immediate and long-term health. The preterm infants especially those with very-low-birth-weight experience disruptive pregnancies, rapid vaginal or Caesarean births, are separated from mothers, and are often treated with antibiotics that are leading causes of dysbiosis and altered gut microbiota [3]. In turn, the gut dysbiosis has been associated with behavioral abnormalities typical of autism. It has been suggested that one of the factors that can ameliorate the dysbiosis is feeding the breast milk [21].

Both the colostrum and the human milk provide several biologically active milk-born proteins important for maintaining gut health and controlling microbial ecosystem [31-33]. Both colostrum and milk also contain important quantities of biologically active milk-borne peptides and proteins known to stimulate the offspring's immune system, digestion and absorption of nutritional elements, development of endogenous defense mechanisms against bacteria, fungi and viruses, prebiotic effects, and others [31-33]. Thus eliminating nursing in preterm infants as well as during infection in term infants may have negative consequences on both immediate and down-stream health. The mother's milk contains 700 species of bacteria and is an important source of *Lactobacillus* and *Bifidobacterium* during nursing. Furthermore, breast-fed vs. formula-fed infants present very different trajectories of microbiota development [34]. It has been suggested that nursing inhibits pathogenic bacteria, and that suboptimal breast-feeding may increase risks of ASD [34,35] and IBD [36].

The premature infant's formulas are based on either cow's milk or soy. While human milk contains 1.4 mg/ml LF, cow's formula contains 0.1 mg/ml and soy based formulas do not contain LF [37]. Even when formulas are supplemented with LF concentration at 0.1 mg/ml, bacterial flora did not contain beneficial *Bifidobacterium species*; LF at 1 mg/ml was able to establish this beneficial bacterial species in half of the infants, but later than in breastfed infants [38]. However, animal data suggest that LF supplementation at high dosage may actually exert detrimental effects as seen in preterm pigs supplemented with a high dose of bovine LF, urging caution [39]. An alternative to formula-feeding, in case when mother milk is unavailable or infant stays in intensive care neonatal units, is donated human milk stored in human milk banks. Freezing at -20°C is the most common way to preserve donor milk. However, frozen storage can reduce nutritional and immunological quality of some milk components [40-43]. Unfortunately, freezing decreases concentration of LF [44]. Still, taking into consideration all these factors, properties of human milk, even frozen and stored, outstrip formula feeding.

Interestingly, donor milk usage in the USA, in level 3 and 4 neonatal ICUs, has increased from 25 to 45% between 2007 and 2011, based on the observation that exclusive human milk diet offers protection against NEC. Several ongoing studies are reevaluating the benefits of exclusive human milk diet vs. formula feeding in preterm infants [45].

## Lactoferrin (LF): Structure-Function Relationship

LF is a sialic acid-rich, iron-binding, bioactive glycoprotein most

abundant in colostrum and milk, but also found in other biological fluids, neutrophile granules and the central nervous system (CNS). Colostral and milk LF binds to its receptors (LFR) in the brush border cells, is absorbed by the epithelial cells of the intestine, where it regulates the proliferation of gut lymphatic follicles and both local and systemic immune response. LF is involved in multiple physiological functions, including antimicrobial, anti-inflammatory, and immunomodulatory; it is also a major transport protein for dietary iron. LF also enhances absorption of nutrients [46] and controls proper composition and proportion of the beneficial gut microbiota [47].

Human milk LF binds to the pathogenic gram-positive and gram-negative bacteria and exerts antimicrobial activity *via* iron depletion and bacterial membrane disruption. It has been shown that diabetes mellitus during pregnancy is associated with altered milk immune factors implicated in modulation of infant immunity; specifically, the colostrum contains lower concentrations of IgA and IgG, and complement C3 protein and presents increased glycosylation of LF [48]. Interestingly, LF is resistant to digestion in the infants' intestines, so its effect is greater in infants than in adults.

LF is critical for both gut and brain development. Interestingly, LF has been found in dopaminergic neurons of substantia nigra and has been shown to be synthesized by human activated microglia [49]. Additionally, LF transcript has been located in pyramidal neurons of the hippocampus of aged mice, suggesting its protective effect from oxidative damage in the brain, However, its level of expression under normal conditions are very low [49].

LF is involved in learning and cognition [50,51]. Interestingly, it has been shown that in newborn calves colostrum LF is transported from plasma to the cerebrospinal fluid (CSF), suggesting it may play an important role in brain functions [52]. Indeed, LF has been shown to suppress psychological stress [53].

Recent proteonomic comparison of human and macaque milk identified 88 differently abundant proteins, including LF that was more abundant in human milk, as well as 524 new human milk proteins [11]. Human native LF and recombinant (rLF) have identical protein sequences, but different glycosylation patterns. Native human LF shows a glycol profile rich in sialic acid, fructose, and mannose, whereas rLF is rich in mannose. While native human LF stimulates IgG and IgE antibody response, rLF is 40-fold less immunogenic and 200-fold less allergenic [12]. Furthermore, the native human and the rLF differed with respect to N-glycosylation sites, consistent with the concept of milk (as well as other proteins) glycosylation profile being both species- and tissue-specific [13]. It has been proposed that these species differences in LF structure are related to the developmental maturity of the organism at birth [11]. One can then pose a question, whether modified LF can replace native human LF in early human nutrition?

## Delayed Maturation of the Gut in Preterm Infants as a Possible Factor Contributing to Autistic Pathology; Role of Lactoferrin

Human infants at term attain certain degree of gut maturity and readiness to make a transition from nutrients present in the mother's blood to the external supply of undigested nutrition. What happens to the preterm infants, lacking several critical weeks of gut development? As already pointed out, preterm infants experience gastrointestinal dysfunctions, sepsis, and NEC that result in altered gut microbiota and dysbiosis [21].

Similarly, children with ASD frequently present a variety of GI symptoms, which include constipation, diarrhea, abdominal pain, gastroesophageal reflux, and vomiting. The prevalence of GI symptoms as high as 23 -70% has been reported in ASD children [54]. The bacterial theory of autism posits that the GI symptoms observed in autism are associated with changes in the microbial composition in the gut and that these changes could be involved in the pathogenesis or progression of the disease. And indeed, several lines of evidence, including the clinical studies seem to support alterations of gut microbiota in autism [3]. Importantly, age-adjusted increase in the prevalence of IBD, based on the case-control studies has been recently reported in patients with autism [20].

It is important to realize, that the intactness of the gut-blood barrier is crucial for maintaining the composition of gut microbiota, and that any damage to that barrier results in changes in its composition. It has been suggested that altered gut microbiota in some ASD children (late onset) may be due to disruption of indigenous microbiota by certain antimicrobial drugs [55]. The *Clostridial* species emerged as the most likely candidate, since it has been implicated in diarrhea diseases of humans and animals. This hypothesis is supported by the reports of increased intestinal permeability [56-58] in children with autism. Treatment with antibiotics is associated with increased intestinal colonization with a number of opportunistic bacteria, including *C. difficile*. Interestingly, the animal studies are in support of human observations; they further suggest that the changes in microbiota may be sex-dependent and more significant in males [59,60]. In summary, there is consensus on the increase of both IBD prevalence [20] and a shift from health promoting bacteria to spore-producing, antibiotic resistant, neurotoxins-producing bacteria in autism.

The human gut, sterile at birth, is first colonized by bacteria during birth and the postpartum period by facultative and anaerobic bacteria, *Lactobacillus* and *Bifidobacterium*, derived from mother's vaginal and fecal bacteria. This first gut colonization is disrupted in children derived by C-sections, as is the case in premature births, when gut is first colonized by predominantly skin bacteria. It has been suggested that the recent increase in C-sections may contribute to reduced bacterial diversity of gut microbiota resulting in 'microbial deprivation syndrome", that is insufficient diversity of microbial stimulation resulting in the abnormal immune system and the CNS maturation [61].

The second gut colonization occurs around the time of weaning when the digestive system adjusts to the switch from mother's milk to solids. The first microbiota, dominated by *Actinobacteria* and *Proteobacteria* are gradually replaced by strict anaerobic members of *Bacteroidetes* and *Firmicutes*. The individual gut profiles are established by the end of the first year, and the adult profiles by 3-5 years [62].

Following weaning the diversity of microbiota increases with a relative increase in *Bacteriodes* and *Clostridium* species [63]. The gut colonization following weaning is associated with changes in the GIT structure and function leading to a temporary drop in gut

permeability, a phenomenon known as gut closure [6]. The timing of gut closure is very critical for the maturation of the GIT, as the process regulates the integrity of gut microbiota and thus protects against environmental insults, including bacterial translocation. The timing of this closure is delayed in preterm animals and in intrauterine growth retarded (IUGR) animals [63]. In preterm infants there is reduced diversity of bacterial species and a higher levels of *Bifidobacterium* and *Bacteroides* [62]. Importantly, there is some evidence pointing to incomplete gut closure in autism resulting in increased gut permeability [58,64,65].

The gut microbiota is directly involved in the development and regulation of GIT structure and function. It participates in the development of intestinal mucosa, and mucosal immunity [66]. It is also involved in many functions, such as, the regulation of epithelial barrier integrity, gut motility, digestion and metabolism, and production of hormones and some vitamins [59].

What happens to the early colonization in preterm infants, in which the gut lining is developmentally delayed? In preterm infants the early colonization is affected by a number of factors such as fast vaginal/C-section delivery, formula feeding, antibiotics exposure etc. Premature epithelium will also differ in gut-microbiota interplay [67]. Importantly, this process is affected by the developmental maturity of not only gut, but also gut-associated lymphoid tissue (GALT), and the interplay with the microbiota. GALT, as the mucosal-associated lymphoid tissue, plays a crucial role in maintaining the balance between inside of the body and the outside (intestine lumen). GALT maintains the anti-inflammatory environment in the intestines, which is necessary for mucosal tolerance [68]. During the development, GALT must learn to differentiate between commensal bacteria or food-derived antigens and the pathogens [69].

In preterm infants both the gut and the immune system are underdeveloped, the latter results in abnormal immune response to commensal bacteria. Instead of the immune tolerance, the immune response develops, that eventually leads to the bacterial penetration through the disrupted gut epithelium and subsequent epithelial injury, which in some cases may lead to NEC [70]. Also, the lack of milk-derived secretory IgA may trigger the abnormal immune response, allowing the microbe penetration through the intestine wall. Normally, antigens in the gut lumen form complexes with secretory IgA, that prevent antigen-directed interference with epithelial cells, while allowing sampling by protrusions of the dendritic cells (DCs), underlying the epithelium [71]. DCs with Toll-like receptors (TLRs) recognize the microbial-associated molecular patterns (MAMPs) and release TGBβ and IL-10 maintaining tolerance. Furthermore, antigen- B cell interaction in mesenteric lymph nodes results in their differentiation to plasma cells producing IgA [55]. Gut maturation also includes the proper formation of tight junctions, between the epithelial cells. Abnormal, or insufficient microflora may reduce the maturation of epithelial barrier that in turn results in "leaky gut", allowing the antigens penetration, and evoking the immune response [71,72]. Interaction of abnormal microbiota with underdeveloped GALT in preterm infants interferes with the immune balance (pro- and anti-inflammatory) resulting in inadequate immune response that disrupts normal gut development. This in turn lead to later malfunction of GALT and may result in developing allergic or autoimmune-based diseases, such as Crohn's disease, or diabetes type-I later in age [73,67]. LF is critical for gut development. Animal experiments have shown that LF inhibits apoptosis, regulating both TGF-β, and caspase-3 expression, and stimulates both the proliferation and differentiation of the epithelial cells in the intestines, increases the villus height, and the expression of the brush-border specific enzymes [74,75]. LF stimulates the growth of gut-associated lymphatic follicles, suggesting its beneficial role in premature infants [76]. LF controls the composition and proportion of the gut microbiota that in turn affect gut development [76].

## Possible Role of Lactoferrin in Brain Development

LF has been implicated in the neurodevelopment [77]. It has been reported that LF administered into the intestine [78], as well as LF absorbed systemically from colostrum are transported into the CSF [79] *via* receptor-mediated mechanism [52]. LF receptors have been detected in brain endothelial capillary cells [80]. These results suggest lactoferrin's involvement in brain maturation.

Diet is a key modulator of the bidirectional signaling pathways between the gut and brain that underlie neurodevelopment, and LF activity may be mediated through gut-brain axis and gut synthesized factors [81], such as BDNF. BDNF, critical for brain growth and maturation, is developmentally regulated [3]. The levels of BDNF in human cord blood increase with the gestation and beyond the first week of age [82]. Furthermore, lower BDNF levels have been observed in the umbilical cord blood of premature than in the full-term infants [83]. BDNF levels are also altered in autism, with both decreased and increased BDNF levels reported in different ASD subpopulations [3].

In general, LF supplementation results in upregulation of BDNF. Supplementation with bovine LF in nursing piglets (equivalent to 10 month old human infants) has been shown to upregulate BDNF in the duodenum [77] and the hippocampal BDNF synthesis at both the mRNA (1.3 times) and the protein (2.1 times) level. It has been suggested that LF upregulates the BDNF signaling pathways and improves learning and memory as shown by the results of the eight-arm radial maze testing [51]. In rats with intrauterine growth retardation (IUGR) induced by the maternal dexamethasone treatment during gestation, maternal supplementation with bovine LF during both gestation and lactation upregulated BDNF expression in hippocampus and increased both neuronal density and neurotransmitter levels in the hippocampus at 7 days of age [84]. Thus LF supplementation may be of benefit in preterm infants and ADS cases characterized by lower than normal BDNF levels.

In addition to the BDNF regulation, LF affects both brain structure and behavior. Male piglets given lactoferrin-containing supplements during lactation period showed higher degree of maturation of the internal capsule and cortical tissue and performed better on the spatial T-maze than unsupplemented controls [85]. In a rat model of brain injury from cerebral hypoxia-ischemia (HI) which has a high incidence in premature infants, maternal LF supplementation through lactation reduced both short- and the long-term brain injury at both metabolic and microstructure level in rat pups at 24-28 weeks human age equivalent [86].

Additionally, LF administered to rats during late postnatal/ early post weaning period exerted behavioral effects that included

less risky behavior and faster escape responses, and sex-dependent water immobility/escape response [50]. LF has been also shown to exert a suppressive effect on distress induced by maternal separation in rat pups [87] and on psychological distress under conditions of moderate stress in adult rats perhaps by activation of endogenous opioidergic system [53]. Data derived from both human and animal studies suggest beneficial effect of LF supplementation on brain biochemistry and behavior, although further insight is needed into LF effect in specific subpopulations of preterm infants and toddlers with suspected autism, before the beneficial action of LF in preventing ASD is accepted.

## Fecal LF as Diagnostic Marker in IBD

Based on several reports it can be concluded that fecal LF is a sensitive and a specific marker for gut inflammation in chronic IBD [88,89]. It can detect intestinal inflammation even in patients with normal CRP, while normal fecal LF excludes the intestinal inflammation [90]. Recent review using a meta-analysis involving seven studies concluded that fecal LF has high pooled sensitivity (0.82) and specificity (0.95) especially in chronic inflammation [91].

Data on fecal LF in autism is very limited. Elevated fecal LF has been reported in 24 percent of autistic stools [92] supporting the need for further exploration of fecal LF as a useful marker in ASD. Interestingly, results of another recent study, evaluating the duodenal biopsies as well as fecal LF in children support the notion that gastrointestinal pathology in ASD is similar to controls [93]. In light of the above, fecal LF could be used as an early diagnostic marker in autistic children with gastrointestinal problems. Elevation in fecal LF would identify those children that can benefit from early dietary intervention.

## Beneficial Role of Breast Feeding and Native Human LF as Autism-Preventing Strategies in Preterm Infants and Existing Gut Targeting Therapies in Autism

The gut microbiota undergoes changes throughout the life span, adjusting to different age-specific requirements of the organism, with diet being one of the key regulatory mechanisms. Age-specific intestinal dysbiosis, defined as increased proportion of harmful to beneficial bacteria, has been shown to precede the NEC in intensive care nurseries and is linked to long-term psychomotor disabilities in very-low-birth-weight infants. Intestinal dysbiosis has also been established as a mediator of both IBD and neurodevelopmental disorders, such as autism [94].

Consequently, dietary intervention can be used to remodel microbiota's profile especially during the critical developmental period, when the intestinal microbiota is still being established. This period coincides with emergence of the autistic symptoms and the onset of regressive types of autism. Therefore, there is an open window of opportunity to remodel gut microbiota in preterm infants as means of ASD prevention. Existing therapies targeting the gut microbiota in autism include dietary restrictions, supplementations, immune therapies, antibiotics, prebiotics and probiotics, and fecal microbiota transplantation (FTM), with future plans including development of gut bacteria-specific vaccines [3]. Recently, therapies targeting directly the gut microbiota composition with probiotics are emerging as a viable strategy in the treatment of the CNS disorders [95]. The probiotics, defined as dietary supplements containing live bacteria, have been shown beneficial also in IBD [96]. Preclinical studies of selected probiotics in healthy volunteers [97] have provided encouraging results for further studies exploring the concept of microbial targeting under pathological conditions. Use of probiotics in autism has been reviewed by Critchfield and colleagues [98]; *Bifidobacterium* and *Lactobacillus* are the main genera showing beneficial effects.

However, all these therapies are targeting already established autism cases, following the critical period of gut and brain development. There is a need to develop dietary therapies for the premature infants and to reestablish age-specific composition of microflora early during development.

As discussed, autism may be associated with prematurity, and the premature infants are fed formulas, rather than breast milk. Importantly, clinical studies indicate a relationship between the lack of breastfeeding in premature infants and development of ASD [99]. Other studies support the notion that breastfeeding may be associated with lower risk of Crohn's disease [36,100-102] and ulcerative colitis [36,101].

LF in the breast milk has been proposed to provide a protection against NEC, intestinal allergy, and bacterial gastroenteritis in preterm infants over two decades ago [103]. Recently this issue has been revisited by clinical trials on efficacy and safety of LF with respect to neonatal sepsis and NEC [104-106]. Supplementation of infant formulas with bovine LF in preterm infants has been shown to reduce the late-onset sepsis [105], but had no effect on NEC [107,108]. However in a most recent randomized clinical trial, bovine LF supplementation has been found effective in preventing NEC [109].

In view of native human LF being structurally unique, both with respect to the peptide composition and glycosylation pattern that are intimately related to its function in the developing humans, more studies are required to re-examine its beneficial function in preterm infants and possible preventive role in autism.

## Conclusion

Data derived from clinical observations support the link between prematurity and GI abnormalities in autism. Several specific points emerged from the reviewed data, and are summarized below: (1) increased incidence of premature births accompanied by a higher survival rate of preterm infants is associated with an increase in GI problems and prevalence of IBD also observed in ASD, suggesting that prematurity and associated delay in gut maturation may be contributing factors to autism; (2) elimination and substitution of the breastfeeding in premature infants with formulas containing altered composition of bioactive factors, such as LF, may alter gut microbiota, delay gut maturation, affect the gut-brain axis and brain development contributing to neuropsychiatric pathologies including ASD; (3) the supplementation of infant formulas with bovine and/or transgenic LF has to be reexamined in view of the species specificity of this glycoprotein. While breastfeeding, frozen human milk, and perhaps native human lactoferrin should be promoted as autism-preventing strategies in premature infants, further studies are needed

to reevaluate supplementation of infant formula with bovine and recombinant human LF.

## References

1. Erickson CA, Stigler KA, Corkins MR, Posey DJ, Fitzgerald JF, McDougle CJ. Gastrointestinal factors in autistic disorder: a critical review. J Autism Dev Disord. 2005; 35: 713-727.

2. Michielan A, D'Incà R. Intestinal Permeability in Inflammatory Bowel Disease: Pathogenesis, Clinical Evaluation, and Therapy of Leaky Gut. Mediators Inflamm. 2015: 628157.

3. Sajdel-Sulkowska EM, Bialy M, Cudnoch-Jedrzejewska. Altered BDNF levels, "leaky gut" and abnormal gut microbiome in autism. In: Brain-Derived Neurotrophic Factor (BDNF): therapeutic approaches, role in neuronal development and effects on cognitive health. Nova Science Publishers. 2015; 147-180.

4. Mahoney AD, Minter B, Burch K, Stapel-Wax J. Autism spectrum disorders and prematurity: a review across gestational age subgroups. Adv Neonatal Care. 2013; 13: 247-251.

5. Centers for Disease Control and Prevention. Autism Spectrum Disorder. Data & Statistics, Prevalence, 2015.

6. Sajdel-Sulkowska EM, Zabielski R. Gut microbiota and brain-gut axis in autism – aberrant development of gut-brain communication and reward circuitry. InTech. 2013.

7. Hansen CF, Thymann T, Andersen AD, Holst JJ, Hartmann B, Hilsted L, et al. Rapid gut growth but persistent delay in digestive function in the postnatal period of preterm pigs. Am J Physiol Gastrointest Liver Physiol. 2016; 310: 550-560.

8. Ait-Beelgnaoui A, Durand H, Cartier c, et al. Prevention of gut leakiness by a probiotic treatment leads to attenuated HPA response to an acute psychological stress in rats. Psychoneuroendocrynology. 2012, 37, 1885-1895.

9. Bercik P, Denou E, Collins J, et al. The intestinal microbiota affect central levels of brain-derived neurotrophic factor and behavior in mice. Gastroenterology. 2011, 141, 599-609.

10. Sajdel-Sulkowska EM. Neurotrophins, their receptors and autism: ligand *vs.* receptor abnormalities. In: The molecular basis of autism. Springer SH Fatemi. 2015, 383-392.

11. Beck KL, Weber D, Phinney BS, et al. Comparative proteonomics of human and macaque milk reveals species-specific nutrition during postnatal development. J Proteome Res. 2015, 14, 2143-2157.

12. Almond RJ, Flanagan BF, Antonopoulos A, Haslam SM, Dell A, Kimber I, et al. Differential immunogenicity and allergenicity of native and recombinant human lactoferrins: role of glycosylation. Eur J Immunol. 2013; 43: 170-181.

13. Yu T, Guo C, Wang J, et al. Comprehensive characterization of the site-specific N-glycosylation of wild-type and recombinant human lactoferrin expressed in the milk of transgenic cloned cattle. Glycobiology, 2011, 21, 206-224.

14. Yeargin-Allsopp M, Rice C, Karapurkar T, Doernberg N, Boyle C, Murphy C. Prevalence of autism in a US metropolitan area. JAMA. 2003; 289: 49-55.

15. Bertrand J, Mars A, Boyle C, Bove F, Yeargin-Allsopp M, Decoufle P. Prevalence of autism in a United States population: the Brick Township, New Jersey, investigation. Pediatrics. 2001; 108: 1155-1161.

16. Elsabbagh M, Divan G, Koh YJ, Kim YS, Kauchali S, Marcín C, et al. Global prevalence of autism and other pervasive developmental disorders. Autism Res. 2012; 5: 160-179.

17. Molodecky NA, Soon IS, Rabi DM, Ghali WA, Ferris M, Chernoff G, et al. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. Gastroenterology. 2012; 142: 46-54.

18. Hein R, Köster I, Bollschweiler E, Schubert I. Prevalence of inflammatory bowel disease: estimates for 2010 and trends in Germany from a large insurance-based regional cohort. Scand J Gastroenterol. 2014; 49: 1325-1335.

19. Kappelman MD, Moore KR, Allen JK, Cook SF. Recent trends in the prevalence of Crohn's disease and ulcerative colitis in a commercially insured US population. Dig Dis Sci. 2013; 58: 519-525.

20. Doshi-Velez F, Avillach P, Palmer N, et al. Prevalence of inflammatory bowel disease among patients with autism spectrum disorders. Inflamm Bowel Dis. 2015; 21: 2281-2288.

21. Groer MW, Gregory KE, Louis-Jacques A, Thibeau S, Walker WA. The very low birth weight infant microbiome and childhood health. Birth Defects Res C Embryo Today. 2015; 105: 252-264.

22. Cortese R, Lu L, Yu Y, Ruden D, Claud EC. Epigenome-Microbiome crosstalk: A potential new paradigm influencing neonatal susceptibility to disease. Epigenetics. 2016; 11: 205-215.

23. Sonntag B, Stolze B, Heinecke A, et al. Preterm birth but not mode of delivery is associated with the increased risk of developing inflammatory bowel disease later in life. Inflamm Bowel Dis. 2007; 13: 1385-1390.

24. Khalili H, Ananthakrishnan AN, Higuchi LM, Richter JM, Fuchs CS, Chan AT. Early life factors and risk of inflammatory bowel disease in adulthood. Inflamm Bowel Dis. 2013; 19: 542-547.

25. Bickle Graz M, Newman CJ, Borradori-Tolsa C. (Neurodevelopmental outcome of very premature infants). Rev Med Suisse. 2014; 10: 450-453.

26. Schieve LA, Clayton HB, Durkin MS, Wingate MS, Drews-Botsch C. Comparison of Perinatal Risk Factors Associated with Autism Spectrum Disorder (ASD), Intellectual Disability (ID), and Co-occurring ASD and ID. J Autism Dev Disord. 2015; 45: 2361-2372.

27. Gray PH, Edwards DM, O'Callaghan MJ, Gibbons K. Screening for autism spectrum disorder in very preterm infants during early childhood. Early Hum Dev. 2015; 91: 271-276.

28. Pyhälä R, Hovi P, Lahti M, Sammallahti S, Lahti J, Heinonen K, et al. Very low birth weight, infant growth, and autism-spectrum traits in adulthood. Pediatrics. 2014; 134: 1075-1083.

29. Padilla N, Eklof E, Martensson GE, et al., Poor brain growth in extremely preterm neonates long before the onset of autism spectrum disorder symptoms. Cereb Cortex. 2015.

30. Goldin RL, Matson JL, Matheis M, Jang J. The relationship between premature birth and caregiver first concern in toddlers with utism spectrum disorder: a brief report. Child Neuropsychol. 2015, 11, 1-7.

31. Raikos V, Dassios T. Health-promoting properties of bioactive peptides derived from milk proteins in infant food: a review. Dairy Sci Technol. 2014; 94: 91-101.

32. López-Expósito I, Recio I. Protective effect of milk peptides: antibacterial and antitumor properties. Adv Exp Med Biol. 2008; 606: 271-293.

33. Baldi A, Ioannis P, Chiara P, Eleonora F, Roubini C, Vittorio D. Biological effects of milk proteins and their peptides with emphasis on those related to the gastrointestinal ecosystem. J Dairy Res. 2005; 72: 66-72.

34. Mulle JG, Sharp WG, Cubells JF. The gut microbiome: a new frontier in autism research. Curr Psychiatry Rep. 2013; 15: 337.

35. Johnson S, Hollis C, Kochhar P, Hennessy E, Wolke D, Marlow N. Autism spectrum disorders in extremely preterm children. J Pediatr. 2010; 156: 525-531.

36. Klement E, Cohen RV, Boxman J, Joseph A, Reif S. Breastfeeding and risk of inflammatory bowel disease: a systematic review with meta-analysis. Am J Clin Nutr. 2004; 80: 1342-1352.

37. Satué-Gracia MT, Frankel EN, Rangavajhyala N, German JB. Lactoferrin in infant formulas: effect on oxidation. J Agric Food Chem. 2000; 48: 4984-4990.

38. Roberts AK, Chierici R, Sawatzki G, Hill MJ, Volpato S, Vigi V. Supplementation of an adapted formula with bovine lactoferrin: 1. Effect on the infant faecal flora. Acta Paediatr. 1992; 81: 119-124.

39. Nguyen DN, Jiang P, Stensballe A, et al. Bovine lactoferrin regulates cell survival, apoptosis and inflammation in intestinal epithelial cells and preterm pig intestine. J Proteonomics. 2016.

40. Akinbi H, Meinzen-Derr J, Auer C, Ma Y, Pullum D, Kusano R, et al. Alterations in the host defense properties of human milk following prolonged storage or pasteurization. J Pediatr Gastroenterol Nutr. 2010; 51: 347-352.

41. Bank MR, Kirksey A, West K, Giacoia G. Effect of storage time and temperature on folacin and vitamin C levels in term and preterm human milk. Am J Clin Nutr. 1985; 41: 235-242.

42. Lozan B, Castellote AI, Montes R, LópezSabater MC. Vitamins, fatty acids, and antioxidant capacity stability during storage of freeze dried human milk. Int J Food Sci Nutr. 2014; 65: 703-707.

43. Silvestre D, Miranda M, Muriach M. Frozen breast milk at -20°C and -80°C: a longitudinal study of glutathione peroxidase activity and malondialdehyde concentration. J Hum Lact. 2010; 26: 35-41.

44. Raoof N, Adamkin DH, Radmacher PG, Telang S. Comparison of lactoferrin activity in fresh and stored human milk. J Perinatol. 2016; 36: 207-209.

45. Colaizy TT. Donor human milk for very low birth weights: patterns of usage, outcomes, and unanswered questions. Curr Opin Pediatr. 2015; 27: 172-176.

46. Lönnerdal B. Bioactive proteins in breast milk. J Paediatr Child Health. 2013; 49: 1-7.

47. Artym J, Zimecki M . (The role of lactoferrin in the proper development of newborns). Postepy Hig Med Dosw (Online). 2005; 59: 421-432.

48. Smilowitz JT, Totten SM, Huang J, Grapov D, Durham HA, Lebrilla C, et al. Human milk secretory immunoglobin A and lactoferrin N-glycans are altered in women with gestational diabetes mellitus. J Nutr. 2013; 143: 1906-1912.

49. Fillebeen C, Mitchell V, Dexter D, Benaissa M, Beauvillain J, Spik G, et al. Lactoferrin is synthesized by mouse brain tissue and its expression is enhanced after MPTP treatment. Brain Res Mol Brain Res. 1999; 72: 183-194.

50. Barrett DW, Lane MA, Wittke AJ. Behavioral effects of bovine lactoferrin administration during postnatal development of rats. Biometal. 2014; 27: 1039-1055.

51. Chen Y, Zheng Z, Zhu X, Shi Y, Tian D, Zhao F, et al. Lactoferrin Promotes Early Neurodevelopment and Cognition in Postnatal Piglets by Upregulating the BDNF Signaling Pathway and Polysialylation. Mol Neurobiol. 2015; 52: 256-269.

52. Talukder MJ, Takeuchi T, Harada E. Receptor-mediated transport of lactoferrin into the cerebrospinal fluid via plasma in young calves. J Vet Med Sci. 2003; 65: 957-964.

53. Kamemori N, Takeuchi T, Hayashida K, Harada E. Suppressive effects of milk-derived lactoferrin on psychological stress in adult rats. Brain Res. 2004; 1029: 34-40.

54. Cao X, Lin P, Jiang P, Li C. Characteristics of the gastrointestinal microbiome in children with autism spectrum disorder: a systematic review. Shanghai Arch Psychiatry. 2013; 25: 342-353.

55. Sandler RH, Finegold SM, Bolte ER, et al. Short-term benefit from oral vancomycin treatment of regressive-onset autism. *J Child Neurol*. 2000; 15: 429-435.

56. Sansoneti PJ. To be or not to be a pathogen: that is the mucosally relevantquestion. *Mucosal Immunology.* 2011; 4: 8-14.

57. D'Eufemia P, Celli M, Finocchiaro R, Pacifico L, Viozzi L, Zaccagnini M, et al. Abnormal intestinal permeability in children with autism. Acta Paediatr. 1996; 85: 1076-1079.

58. De Magistris L, Familiari V, Pascotto A, Sapone A, Frolli A, Iardino P, et al. Alterations of the intestinal barrier in patients with autism spectrum disorders and in their first-degree relatives. J Pediatr Gastroenterol Nutr. 2010; 51: 418-424.

59. Grenham S, Clarke G, Cryan JF, Dinan TG. Brain-gut-microbe communication in health and disease. Front Physiol. 2011; 2: 94.

60. De Theije CG, Wopereis H, Ramadan M, van Eijndthoven T, Lambert J, Knol J, et al. Altered gut microbiota and activity in a murine model of autism spectrum disorders. Brain Behav Immun. 2014; 37: 197-206.

61. McDade TW. Early environments and the ecology of inflammation. Proc Natl Acad Sci. USA. 2012; 109 Suppl 2: 17281-17288.

62. Rodríguez JM, Murphy K, Stanton C, Ross RP. The composition of the gut microbiota throughout life, with an emphasis on early life. Microb Ecol Health Dis. 2015; 26: 26050.

63. Mickiewicz M, Zabielski R, Grenier B, et al. Structural and functional development of small intestine in intrauterine growth retarded porcine offspring born to gilts fed diets with differing protein ratios throughout pregnancy. J Physiol Pharmacol. 2012, 63, 225-239.

64. White JF. Intestinal pathophysiology in autism. Exp Biol Med (Maywood). 2003; 228: 639-649.

65. Heberling CA, Dhurjati PS, Sasser M. Hypothesis for a systems connectivity model of Autism Spectrum Disorder Pathogenesis: links to gut bacteria, oxidative stress, and intestinal permeability. Med Hypotheses. 2013; 80: 264-270.

66. Sekirov I, Russell SL, Antunes LC, Finlay BB. Gut microbiota in health and disease. Physiol Rev. 2010; 90: 859-904.

67. Groer MW, Luciano AA, Dishaw LJ, Ashmeade TL, Miller E, Gilbert JA. Development of the preterm infant gut microbiome: a research priority. Microbiome. 2014; 2: 38.

68. Du Pré MF, Samsom JN. Adaptive T-cell responses regulating oral tolerance to protein antigen. Allergy. 2011; 66: 478-490.

69. Bergstrom KS, Sham HP, Zarepour M, Vallance BA. Innate host responses to enteric bacterial pathogens: a balancing act between resistance and tolerance. Cell Microbiol. 2012; 14: 475-484.

70. Hunter CJ, De Plaen IG. Inflammatory signaling in NEC: Role of NF-κB, cytokines and other inflammatory mediators. Pathophysiology. 2014; 21: 55-65.

71. Di Mauro A, Neu J, Riezzo G, Raimondi F, Martinelli D, Francavilla R, et al. Gastrointestinal function development and microbiota. Ital J Pediatr. 2013; 39: 15.

72. Kelly JR, Kennedy PJ, Cryan JF, Dinan TG, Clarke G, Hyland NP. Breaking down the barriers: the gut microbiome, intestinal permability and stress-related psychiatricdisorders. Frontiers in Cellular Neuroscience. 2015; 9: 1-20.

73. Hanaway P. Balance of flora, galt, and mucosal integrity. Altern Ther Health Med. 2006; 12: 52-60.

74. Liao Y, Jiang R, Lönnerdal B. Biochemical and molecular impacts of lactoferrin on small intestinal growth and development during early life. Biochem Cell Biol. 2012; 90: 476-484.

75. Blais A, Fan C, Voisin T, Aattouri N, Dubarry M, Blachier F, et al. Effects of lactoferrin on intestinal epithelial cell growth and differentiation: an *in vivo* and *in vitro* study. Biometals. 2014; 27: 857-874.

76. Artym J, Zimecki M. Milk-derived proteins and peptides in clinical trials. Postepy Hig Med Dosw (Online). 2013; 67: 800-816.

77. Yang C, Zhu X, Liu N, Chen Y, Gan H, Troy FA, et al. Lactoferrin up-regulates intestinal gene expression of brain-derived neurotrophic factors BDNF, UCHL1 and alkaline phosphatase activity to allievate early weaning diarrhea in postnatal piglets. J Nutr Biochem. 2014; 2: 834-842.

78. Harada E, Sugiyama A, Takeuchi T, Sitizyo K, Syuto B, Yajima T, et al. Characteristic transfer of colostral components into cerebrospinal fluid via serum in neonatal pigs. Biol Neonate. 1999; 76: 33-43.

79. Talukder MJ, Takeuchi T, Harada E. Transport of colostral macromolecules into the cerebrospinal fluid via plasma in newborn calves. J Dairy Sci. 2002; 85: 514-524.

80. Huang RQ, Ke WL, Qu YH, Zhu JH, Pei YY, Jiang C. Characterization of lactoferrin receptor in brain endothelial capillary cells and mouse brain. J Biomed Sci. 2007; 14: 121-128.

81. Galland L. The gut microbiome and the brain. J Med Food. 2014; 17: 1261-1272.

82. Rao R, Mashburn CB, Mao J, Wadhwa N, Smith GM, Desai NS. Brain-derived neurotrophic factor in infants <32 weeks gestational age: correlation with antenatal factors and postnatal outcomes. Pediatr Res. 2009; 65: 548-552.

83. Malamitsi-Puchner A, Economou E, Rigopoulou O, Boutsikou T. Perinatal changes of brain-derived neurotrophic factor in pre- and full term neonates. Early Hum Dev. 2004; 76: 17-22.

84. Somm E, Larvaron P, van de Looij Y, Toulotte A, Chatagner A, Faure M, et al. Protective effects of maternal nutritional supplementation with lactoferrin on growth and brain metabolism. Pediatr Res. 2014; 75: 51-61.

85. Mudd AT, Alexander LS, Berding K, Waworuntu RV, Berg BM, Donovan SM, et al. Dietary prebiotics, milk fat globule membrane, and lactoferrin affects structural neurodevelopment in the young piglet. Front Pediatr. 2016.

86. van de Looij Y, Ginet V, Chatagner A, Toulotte A, Somm E, Hüppi PS, et al. Lactoferrin during lactation protects the immature hypoxic-ischemic rat brain. Ann Clin Transl Neurol. 2014; 1: 955-967.

87. Takeuchi T, Hayashida Ki, Inagaki H, Kuwahara M, Tsubone H, Harada E. Opioid mediated suppressive effect of milk-derived lactoferrin on distress induced by maternal separation in rat pups. Brain Res. 2003; 979: 216-224.

88. Kane SV, Sandborn WJ, Rufo PA, Zholudev A, Boone J, Lyerly D, et al. Fecal lactoferrin is a sensitive and specific marker in identifying intestinal inflammation. Am J Gastroenterol. 2003; 98: 1309-1314.

89. Borkowska A, Liberek A, luczak G, Jankowska A, Plata-Nazar K, Korzon M, et al. Fecal lactoferrin, a marker of intestinal inflammation in children with inflammatory bowel disease. Acta Biochim Pol. 2015; 62: 541-545.

90. Buderus S, Boone JH, Lentze MJ. Fecal Lactoferrin: Reliable Biomarker for Intestinal Inflammation in Pediatric IBD. Gastroenterol Res Pract. 2015; 2015: 578527.

91. Wang Y, Pei F, Wang X, Sun Z, Hu C, Dou H. Diagnostic accuracy of fecal lactoferrin for inflammatory bowel disease: a meta-analysis. Int J Clin Exp Pathol. 2015; 8: 12319-12332.

92. Martirosian G, Ekiel A, Aptekorz M, WiechuÅB, Kazek B, Jankowska-Steifer E, et al. Fecal lactoferrin and Clostridium spp. in stools of autistic children. Anaerobe. 2011; 17: 43-45.

93. Kushak RI, Buie TM, Murray KF, Newburg DS, Chen C, Nestoridi E, et aL. Evaluation of Intestinal Function in Children with Autism and Gastrointestinal Symptoms. J Pediatr Gastroenterol Nutr. 2016; 62: 687-691.

94. Sherman MP, Zaghouani H, Niklas V. Gut microbiota, the immune system, and diet influence the neonatal gut-brain axis. Pediatr Res. 2015; 77: 127-135.

95. Forsythe P, Sudo N, Dinan T, Taylor VH, Bienenstock J. Mood and gut feelings. Brain Behav Immun. 2010; 24: 9-16.

96. Moayyedi P, Ford AC, Talley NJ, Cremonini F, Foxx-Orenstein AE, Brandt LJ, et al. The efficacy of probiotics in the treatment of irritable bowel syndrome: a systematic review. Gut. 2010; 59: 325-332.

97. Messaoudi M, Lalonde R, Violle N, Javelot H, Desor D, Nejdi A, et al. Assessment of psychotropic-like properties of probiotic formulation (Lactobacillus helveticus R0052 and Bifidobacterium longum R0175) in rat and human subjects. Br J Nutr. 2011; 105: 755-764.

98. Critchfield JW, van Hemert S, Ash M, Mulder L, Ashwood P. The potential role of probiotics in the management of childhood autism spectrum disorders. Gastroenterol Res Pract. 2011; 2011: 161358.

99. Goldin RL, Matson JL, Matheis M, Jang J. The relationship between premature birth and caregiver first concern in toddlers with autism spectrum disorder: a brief report. Child Neuropsychol. 2015; 11: 1-7.

100. Koletzko S, Sherman P, Corey M, Griffiths A, Smith C. Role of infant feeding practices in development of Crohn's disease in childhood. BMJ. 1989; 298: 1617-1618.

101. Rigas A, Rigas B, Glassman M, Yen YY, Lan SJ, Petridou E, et al. Breast-feeding and maternal smoking in the etiology of Crohn's disease and ulcerative colitis in childhood. Ann Epidemiol. 1993; 3: 387-392.

102. Thompson NP, Montgomery SM, Wadsworth ME, Pounder RE, Wakefield AJ. Early determinants of inflammatory bowel disease: use of two national longitudinal birth cohorts. Eur J Gastroenterol Hepatol. 2000; 12: 25-30.

103. Walker WA. Breast milk and the prevention of neonatal and preterm gastrointestinal disease states: a new perspective. Zhonghua Min Guo Xiao Er Ke Yi Xue Hui Za Zhi. 1997; 38: 321-331.

104. Lingappan K, Arunachalam A, Pammi M. Lactoferrin and the newborn: current perspectives. Expert Rev Anti Infect Ther. 2013; 11: 695-707.

105. Sharma D, Shastri S. Lactoferrin and neonatology - role in neonatal sepsis and necrotizing enterocolitis: present, past and future. J Matern Fetal Neonatal Med. 2016; 29: 763-770.

106. Manzoni P, Meyer M, Stolfi I, Rinaldi M, Cattani S, Pugni L, et al. Bovine lactoferrin supplementation for prevention of necrotizing enterocolitis in very-low-birth-weight neonates: a randomized clinical trial. Early Human Dev. 2014; 90: 60-65.

107. Venkatesh MP, Abrams SA. Oral lactoferrin for the prevention of sepsis and necrotizing enterocolitis in preterm infants. Cochrane Database Syst Rev. 2010.

108. Pammi M, Abrams SA. Oral lactoferrin for the prevention of sepsis and necrotizing enterocolitis in preterm infants. Cochrane Database Syst Rev. 2015.

109. Manzoni P, Rinaldi M, Cattani S, Pugni L, Romeo MG, Messner H, et al. Bovine lactoferrin supplementation for prevention of late-onset sepsis in very low-birth-weight neonates: a randomized trial. JAMA. 2009; 302: 1421-1428.

Austin J Autism & Relat Disabil - Volume 2 Issue 3 - 2016
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**Citation:** Makowska M, Kasarello K, Bialy M and Sajdel-Sulkowska EM. Autism: "Leaky Gut", Prematurity and Lactoferrin. Austin J Autism & Relat Disabil. 2016; 2(3): 1021.