The Significance of the Enteric Microbiome on the Development of Childhood Disease: A Review of Prebiotic and Probiotic Therapies in Disorders of Childhood

John Slattery[1,2], Derrick F. MacFabe[3] and Richard E. Frye[1,2]

[1]Arkansas Children's Research Institute, Little Rock, AR, USA. [2]Division of Neurology, Department of Pediatrics, University of Arkansas for Medical Sciences, Little Rock, AR, USA. [3]The Kilee Patchell-Evans Autism Research Group, Departments of Psychology (Neuroscience) and Psychiatry, Division of Developmental Disabilities, University of Western Ontario, London, ON, Canada.

ABSTRACT: Recent studies have highlighted the fact that the enteric microbiome, the trillions of microbes that inhabit the human digestive tract, has a significant effect on health and disease. Methods for manipulating the enteric microbiome, particularly through probiotics and microbial ecosystem transplantation, have undergone some study in clinical trials. We review some of the evidence for microbiome alteration in relation to childhood disease and discuss the clinical trials that have examined the manipulation of the microbiome in an effort to prevent or treat childhood disease with a primary focus on probiotics, prebiotics, and/or synbiotics (ie, probiotics + prebiotics). Studies show that alterations in the microbiome may be a consequence of events occurring during infancy and/or childhood such as prematurity, C-sections, and nosocomial infections. In addition, certain childhood diseases have been associated with microbiome alterations, namely necrotizing enterocolitis, infantile colic, asthma, atopic disease, gastrointestinal disease, diabetes, malnutrition, mood/anxiety disorders, and autism spectrum disorders. Treatment studies suggest that probiotics are potentially protective against the development of some of these diseases. Timing and duration of treatment, the optimal probiotic strain(s), and factors that may alter the composition and function of the microbiome are still in need of further research. Other treatments such as prebiotics, fecal microbial transplantation, and antibiotics have limited evidence. Future translational work, *in vitro* models, long-term and follow-up studies, and guidelines for the composition and viability of probiotic and microbial therapies need to be developed. Overall, there is promising evidence that manipulating the microbiome with probiotics early in life can help prevent or reduce the severity of some childhood diseases, but further research is needed to elucidate biological mechanisms and determine optimal treatments.

KEYWORDS: allergies, asthma, autism spectrum disorders, colic, diabetes, antibiotic-associated diarrhea, eczema, fetal microbiome, irritable bowel syndrome, microbiome, necrotizing enterocolitis, prematurity, probiotics, fecal transplantation, short-chain fatty acids

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CORRESPONDENCE: REFrye@uams.edu

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Introduction

> "*Let food be thy medicine, and medicine be thy food.*
> *Everything in excess is opposed to nature.*
> *All disease begins in the gut.*"
>
> – Hippocrates

The human body houses a diverse ecosystem of bacteria, fungi, viruses, protozoa, and their genomes, which is collectively referred as the microbiome. The human body houses 10 times more microbial cells than human cells, with their genetic material outnumbering human DNA by 100 to 1.[1–3] The microbiome has been referred to as the "forgotten organ," and numerous lines of research have implicated disruptions in the microbiota that inhabit the gut, skin, oral cavity, lung, vagina, and placenta[4] have been implicated across varied human disease processes. Of particular focus in human disease has been the disruption of the enteric (gut) microbiota due to its relationship to metabolic and immune function, as well as vitamin creation and epigenetic programming.[5]

This paper reviews the significance of the microbiome in childhood. We start by reviewing the significance of the enteric microbiome, and then review the temporal development of the microbiome during early life. We then review the evidence for disruption of the microbiome in specific childhood diseases as well as the evidence for treatments targeting the microbiome that are related to the prevention or treatment of childhood diseases. The evidence for microbiome changes related to specific childhood diseases are provided in Table 1, including the important studies and a summary for the evidence for each disease if any consistent findings are present across studies.

Table 1. Microbiome disruption by condition summary.

CONDITION	RELEVANT FINDINGS
Prematurity	↑ Proteobacteria ↓ Microbial diversity
Necrotizing enterocolitis	Blooms of *Proteobacteria* prior to disease onset
Sepsis	Altered microbiota structure and composition prior to disease onset has been reported, but specific microbiota reported is inconsistent across studies
Colic	Decreased microbial diversity and increased anaerobic bacteria
Malnutrition	Anaerobic depletion, early dysbiosis, and intestinal pathogenic overabundance with decreased bacterial diversity
Eczema	Early colonization with opportunistic species may be important in disease initiation
Allergies	↓ Species diversity
Asthma	No clear pattern
Inflammatory bowel disease	Data is sparse, no consistent pattern
Type I diabetes	↑ *Bacteroidetes:Firmicutes* ratios, ↑ *Clostridia* species ↓ Butyrate-producing bacteria ↓ Bacterial diversity ↓ Community stability Alterations in the microbiome seem to precede disease onset
Type II diabetes and obesity	↑ *Firmicutes:Bacteroidetes* ratio ↑ SCFAs
Autism spectrum disorder	↑ *Clostridial* species ↑ *Suttetrella* and *Desulfovibrio* species

Notes: Limitations of microbiome studies are related to unknowns if microbiota changes occur prior to disease onset, prodromal periods of disease, active disease processes. For the most part it is unknown if microbiota changes are causal to disease or are merely associated with most diseases.

Significance of the Enteric Microbiome

There is a growing body of clinical and basic science evidence that the enteric microbiome (the trillions of bacteria and their collective genomes that inhabit the human digestive tract) and/or their metabolic end products affects immune and metabolic function[6–8] as well as having modulatory effects on gene expression through epigenetic mechanisms.[5,9] The enteric microbiome can lead to alterations in host physiology and a wide array of disease processes that affect the gut from disorders such as inflammatory bowel disease (IBD) to conditions that are regarded as "autoimmune", such as type I diabetes, rheumatoid arthritis, and multiple sclerosis, as well as conditions such as obesity, metabolic syndrome, cardiovascular disease, and some malignancies[10–12] have also been associates with microbiota disruption.

The microbiome produces several mediators such as lipopolysaccharides, peptidoglycans, short-chain fatty acids (SCFAs), and gaseous molecules (ie, nitric oxide), which influence host physiology depending upon the dose, developmental time period, and tissue type.[13–15] For example, *Clostridia* spp. are producers of the SCFA propionic acid (PPA)[16,17] following the fermentation of dietary carbohydrates and proteins. PPA as well as other SCFA bacterial fermentation products (eg, butyric and acetic acid) are compounds that are increasingly recognized as being important in the maintenance of health and have been implicated as possible contributing or protective factors for certain disease processes.[17–19] For example, PPA can modulate cell signaling (eg, specific free fatty acid G protein coupled receptors),[20,21] cell–cell interactions (eg, gap junctions),[22] gene expression (eg, histone deacetylase inhibition),[23,24] immune function,[25] and neurotransmitter synthesis and release,[26] as well as influence mitochondrial[27] and lipid[28,29] metabolism. Poor fiber intake can lead to inflammatory conditions via alteration in the enteric microbiota, which results in the decreased production of the SCFA butyrate.[30–32]

Other microbiota-derived metabolites that play wide and significant roles in both host health and human disease include tryptophan, an essential amino acid found in a variety of foods, and the metabolic products of tryptophan, indole, and its derivatives. Indole derivatives such as indole-2-propionate (IPA) and indoxyl sulfate can have profound effects on the integrity of the intestinal barrier, oxidative stress, renal function, vascular health, and immunity and can have antioxidant and neuroprotective effects. Many of the effects of tryptophan and its derivatives depend on the microbiome, as different microbes have different metabolic pathways to metabolize tryptophan into different indole derivatives.[33,34] Furthermore, the gut microbiota has also been shown to regulate serotonin production, of which tryptophan is a precursor.[35] It has been hypothesized that this type of metabolic disruption could potentially lead to a variety of neuropsychiatric conditions via disruption in the microbiota-gut-brain connection. Further explanations for how tryptophan metabolism may be disrupted by the host microbiota include the fact that SCFAs produced by the human microbiota promote transcription of tryptophan hydroxylase 1, which is the rate-limiting enzyme in the production of serotonin.[36–39] By regulating tryptophan metabolism, the microbiome also can regulate the kynurenine pathway, which can have significant effects on brain function and potentially contribute to psychiatric disease.[40]

Other significant metabolites derived from the host microbiota include tyrosine and phenylalanine metabolites and others derived from the diet. These may also impact the immune and endocrine systems, affect intestinal barrier function, cause anti-inflammatory or inflammatory processes, as well as regulate oxidative and nitrosative stress.[33]

Development of the Microbiome: Pre and Postnatal Time Periods

It has been long believed that the womb is a sterile environment and that the fetus is exposed to microbes only upon delivery. However, studies of both premature and term birth

demonstrate that both the placenta[41] and meconium[42] have their own microbiome(s), suggesting that the fetus is exposed to bacteria in the uterus. Studies have suggested that the maternal diet has a large role in determining this fetal microbiome and that both inflammatory and metabolic mediators produced by the microbiome may affect the fetus directly or indirectly (eg, placental disruption). In fact, animal studies have suggested that the maternal microbiome may have a large influence on causing gestational diabetes.[43] Thus, the consequence of routine treatments during pregnancy, such as antibiotics and proton pump inhibitors,[44] may not always be benign and could have a significant effect not only on the maternal microbiome but also on the fetus' microbiome and subsequent health-related outcomes. Indeed, the time and mode of delivery, maternal age, diet, hospitalization, body mass index (BMI), smoking status and socioeconomic status, breastfeeding, and antibiotic use, all influence the development of the infant microbiome.[45] Although these studies are only preliminary, they point to an important set of factors that may be manipulated or controlled to improve maternal health, fetal outcomes, and childhood disease.

Emerging data from large-scale ongoing longitudinal studies in Scandinavia (NOMIC),[46,47] Canada (CHILD),[48,49] and studies that show a contrast between developed versus developing countries (eg, MAL-ED)[50,51] reveal the effect of birth practices, early nutrition, and antibiotic exposure on early alterations of the developing microbiome as a predictor and probable contributor of later health and disease. Indeed, the excellent work by Gordon and Knight related to disruption of the microbiota in childhood malnutrition in the developing world is of keen interest and is highly noteworthy and revolutionary.[52] Collectively, these studies strongly suggest that certain practices early in life lead to alteration in the child's gut microbiome with resultant effects on immune and metabolic development, leading to later chronic metabolic and immune disorders and reduced efficacy of vaccination regimes.

Other studies have examined the development of the microbiome during early and late childhood. The infant gut microbiota communities undergo a complex temporal transition and change in complexity and is easily influenced by diet, medications, and environmental factors.[53] Long-term stability in many microbiome species starts at about 2 years of age, with greater microbiome stability associated with higher species diversity.[54] Microbiomes of children contain some of the same core species, most notably *Bacteroidetes* spp. and *Firmicutes* spp., as adult microbiomes; further, the microbiome of the child appears to resemble that of an adult by 3 years of life.[54]

A study comparing the enteric microbiome in preadolescent school-aged children to healthy adults revealed some interesting differences.[55] While children demonstrated more *Bifidobacterium* spp., *Faecalibacterium* spp., and *Lachnospiraceae* spp., adults harbored more *Bacteroides* spp. The enteric microbiome derived from children was enriched with genes involved in vitamin B12 and folate synthesis, while the adult enteric microbiome was enriched with genes involved in mitochondrial function and lipopolysaccharide (LPS) biosynthesis. Interestingly, while both children and adults demonstrated enrichment of genes involved in amino acid metabolism, the specific amino acids involved differed between children and adults, with children demonstrating enrichment of genes involved in phenylalanine, tyrosine, tryptophan, and lysine biosynthesis and adults demonstrating enrichment of genes involved in alanine, aspartate, and glutamate metabolism.

Infantile Risk Factors and Diseases Associated with Microbiome Perturbation

Prematurity. As compared to term infants, premature infants show different microbes in their amniotic fluid, placenta, and meconium.[41,42,56–58] In fact, one study linked premature birth to *Lactobacillus*-poor vaginal microbiota.[59] *Gardnerella* spp. and *Ureaplasma* spp. were increased in *Lactobacillus*-poor vaginal microbiota. However, other studies have been unable to confirm differences in vaginal microbiota in women delivering at term versus premature.[60] Further, studies have shown that the premature infant's microbiota composition and function is compromised and shows delays and/or deficiencies in development and that this is complicated by perinatal antibiotic usage, which may have consequences for later health of the infant.[61,62]

Although conceptually promising,[58] the few studies that have looked at the effect of prenatal probiotic supplementation have reported mixed results. A Norwegian study of 950 cases and 17,938 controls studied the intake of milk-based products containing the probiotic *Lactobacilli* using a food-frequency questionnaire as a measure of consumption. Intake of milk-based probiotic products was associated with a reduced risk of spontaneous preterm delivery in a dose-dependent manner.[63] However, in another study of 104 cases and 200 controls, *Lactobacillus* in early pregnancy (median exposure time was 5.2 weeks gestation) with a median length of exposure of 4 days (range 1–90 days) was not associated with a decreased risk of preterm birth.[64] A study of 147 preterm infants randomized to one of the three *Bifidobacterium* supplement regimens during the first 7 days of life and treated for 4–6 weeks, depending on gestational age at entry, showed no benefits of therapy related to postnatal growth parameters, although the probiotic supplementation was considered safe with no adverse events reported in this very high risk population.[65] Lastly, in a study of 2,491 women, 13.7% of whom used self-prescribed probiotics, no improvement in pregnancy outcomes was found.[66] Interestingly, in light of the mixed data on these probiotic treatment trials, emerging studies in east Africa have suggested that the development and cultivation of locally grown fermented foods and probiotic cultures may be an emerging alternative prophylactic practice aimed at providing protection for illness or toxin exposure and an alternative approach to therapeutic administration of probiotics and/or prebiotics.[67–70]

Necrotizing enterocolitis. Necrotizing enterocolitis (NEC) is a potentially deadly disease that primarily affects premature neonates. In many cases, NEC results in serious complications including bowel necrosis and perforation, colectomy, and short bowel syndrome as well as associated neurological and neurodevelopmental abnormalties.[71,72] Previously, NEC was viewed as a primary infectious disease, but understanding the microbiome in more detail and microbiota dysbiosis has shed light on the complex nature of this disorder. The microbiome differences associated with NEC are complex. Although the same bacterial species are found in both NEC and in non-NEC neonates, neonates with NEC have changes in the overall structure of the microbiome, involving differences in bacterial diversity and microbiome complexity and fluidity.[73]

The microbial ecology of the neonate who develops NEC differs from that of control infants.[74–76] The phylum *Proteobacteria* appears to be overrepresented before the development of NEC.[74–81] Of interest, this particular phylum contains numerous gram-negative pathogens with high levels of cell-wall LPSs. There is also increased production of SCFAs, in particular PPA,[82] which has been substantiated in an animal model of NEC[83] and has been proposed as a contributor to NEC-associated neurodevelopmental conditions including movement disorders, seizure, and developmental delay.[17,84] These microbes and their metabolites are also highly prevalent in other disease entities such as IBD, in which blooms are seen prior to exacerbations of the inflammation.[85] This blooming pattern, which preceding disease manifestation, is also found in very low birth weight infants with NEC.[80,86] Interestingly, Bucher et al.[87] found that, on average, there was almost twice as much bacterial DNA content in the intestinal walls in infants with acute NEC than in the same infants after NEC had resolved, which the authors concluded as underscoring the relevance of invasive bacterial species, especially Gram-negative species, translocating across the intestinal barrier as being crucial to the pathogenesis of NEC.

NEC is associated with factors that influence the microbiome. For example, prolonged antibiotic use decreases microbiome diversity and increases the risk of NEC[88]; proton pump inhibitors, which have been shown to cause polymicrobial small-bowel bacterial overgrowth and *Clostridium difficile* infection,[44] are associated with increased risk of NEC[89–91]; and breast feeding, which provides beneficial bacteria[92–94] and essential prebiotics,[95,96] decreases the risk of NEC.[97] Conversely, long-term hospitalization, which may introduce nosocomial microflora, may alter the microbiome, increasing NEC-associated complications.[71]

Probiotics have been the subject of significant study for the treatment of NEC.[98] A recent systematic review concluded that there are some encouraging randomized controlled trials (RCTs) that supported evidence for probiotics reducing the severity and mortality of NEC.[99] A more recent Cochrane Review, which included 24 clinical trials, found a significant effect of probiotics in reducing the incidence of mortality and severe NEC.[100] The study noted variability in birth weight, gestational age, timing, dose, formulation of the probiotics, and feeding regimens across the studies. Although significant effects were found in very low birth weight neonates (VLBW; <1500 g at birth), these effects were not significant for extremely low birth weight neonates (<1000 g at birth). Probiotic preparations that contained *Lactobacillus* alone or a probiotic mixture demonstrated significant protection against NEC, while probiotics that contained only *Bifidobacterium* or *Saccharomyces boulardii* alone were not effective. A meta-analysis of probiotic administration in NEC, which analyzed 20 RCTs involving 5,982 preterm VLBW infants, was published by Lau and Chamberlain.[101] This review conducted a comprehensive literature search between 1966 and 2014 across PubMed, Cochrane Central Registry of Controlled Trials, and Google Scholar, and assessed the incidence of NEC, sepsis, overall mortality, and time to reach full enteral feeds. The review found that probiotics reduced the risk of NEC, overall mortality, sepsis, and time to full enteral feeds by 49%, 27%, 8.1%, and 1.2 days, respectively. The authors concluded that the use of probiotics in preterm VLBW infants is associated with a significant reduction in NEC risk as well as overall mortality in this population, although the authors caution that more research is needed. Another systematic review and meta-analysis published by Aceti et al.[102] found that probiotics had an overall protective effect on NEC in preterm infants. A recent systematic review by Baucelles et al.[103] also found positive effects of probiotics in premature VLBW infants, with combinations of three probiotics providing better benefits. A recent meta-analysis of observational studies also concluded that prophylactic probiotic supplementation reduced the risk of NEC and mortality in preterm infants.[104]

Prebiotics have also been studied for the prevention of NEC. A meta-analysis in 2013 examined five prebiotic trials using oligosaccharide supplementation in preterm infants. Although this prebiotic was found to increase *Bifidobacteria* and reduce stool viscosity and pH, there was no difference in the incidence of NEC.[105] In contrast, a more recent study showed that supplementation with an oligosaccharide prebiotic in exclusively breast-fed, VLBW, preterm infants reduced the incidence of NEC.[106] Lastly, a more recent study found that prebiotic supplementation with inulin decreased the incidence of NEC in VLBW infants only when given with the probiotic *Bifidobacterium lactis*.[107]

Thus, although overall there is promising evidence to suggest that prebiotics and probiotics have a role in decreasing the incidence of NEC, the precise composition of the organism(s) in the probiotic, the exact characteristics of the infants (term vs. premature vs. VLBW), the associated enteral feeding (breast milk vs. formula), and the potential long-term complications require further large-scale longitudinal studies in order to determine the optimal treatment and the target population best suited for these therapies.[48]

Nosocomial infections. One study examined the stool in a small number (N = 6) of VLBW premature infants. Stool obtained early in life from neonates who eventually developed late-onset sepsis (LOS) was found to be low in diversity and contained a predominance of *Staphylococcus*, while the neonates who did not develop sepsis demonstrated a more diverse microbiome with a predominance of *Clostridium*, *Klebsiella,* and *Veillonella*.[108] Further, a prospective case–control trial of preterm infants found that infants who developed LOS showed a stable decrease in *Bifidobacteria* with a dynamic change in *Proteobacteria,* being lower prior to diagnosis of sepsis with a bloom around the time of diagnosis. The authors hypothesize that this pattern may result in an excessive immune response that could potentially compromise intestinal barrier function and propose that permeability of the intestinal barrier may result and lead to translocation of intestinal bacteria and be mechanistic in the development of sepsis.[109] Although there does not appear to be a microbial composition that can predict LOS, the source of microbes that results in sepsis may originate from the gut as a consequence of a failure to produce a mature microbiota profile and seems to be responsible for disease-related pathology.[110,111]

Several studies, particularly those that have examined the effect of probiotics on the incidence of NEC, have also examined the effect of probiotic treatments on nosocomial infections. A recent systematic review of RCTs that used probiotics for NEC found that there was no overall benefit of probiotics in preventing sepsis.[100] However, a more recent meta-analysis that included 4,078 more patients and 18 more trials than the previous meta-analysis found a significant effect of probiotic supplementation compared to no probiotics or placebo in LOS.[112] A recent systematic review found that the use of *Lactobacillus reuteri* significantly reduced the risk of LOS.[113] A small study followed 66 premature infants at 0, 2, and 4 weeks and found the probiotic *Bifidobacterium breve* M-16V altered fecal SCFA composition, leading to predictively favorable increase in acetate and decreases in propionate and butyrate.[82] As stated previously, a trial by Dilli et al.[107] found that *B. lactis* with or without inulin decreased the rate of nosocomial sepsis. Jacobs et al.[114] demonstrated that the probiotic combination *Bifidobacterium infantis, Streptococcus thermophilus,* and *B. lactis* significantly reduced NEC of Bell stage 2 or more in very preterm infants but had no effect on LOS. A Phase III clinical trial of *B. breve* found no effect of this probiotic in a multicenter, double-blind, placebo-controlled trial of 1,315 infants on NEC or LOS.[115] One study examined the effect of a probiotic consisting *Lactobacillus casei*, *Lactobacillus acidophilus*, *Bacillus subtilis*, and *Enterococcus faecalis* on preventing critical illness in term infants. The risk of nosocomial pneumonia, but not sepsis, was found to be reduced.[116] A systematic review and meta-analysis by Zhang et al.[117] also demonstrated that in 25 trials of 6,104 neonates, probiotics appeared to be safe and reduced the risk of LOS in neonates in the NICU.

A few studies have examined the prevention of fungal infections. One study found that a probiotic containing *B. infantis*, *Lactobacillus,* and *B. lactis* significantly decreased both stool fungal colonization and invasive infections in VLBW infants.[118] Another study suggested that *S. boulardii* was as effective as nystatin in reducing fungal colonization and invasive infections in VLBW neonates.[119]

Infantile colic. Infantile colic has been associated with reduced *Bifidobacterium* and *Lactobacilli* as well as reduced microbiome diversity and increased *Proteobacteria*, suggesting a particular microbiome signature that may predate colic symptoms.[120] Other studies have found similar results related to abnormal microbial profiles in colicky versus non-colicky infants.[121–123] A recent systematic review and meta-analysis examining treatments for infantile colic identified seven high-quality probiotic clinical trials.[124] Six of these studies utilized *L. reuteri* while one study used a synbiotic mixture of six species of microbiota, not including *L. reuteri*, with the prebiotic fructo-oligosaccharide. Meta-analysis of the six studies demonstrated that *L. reuteri* decreased crying time by about 56 minutes per day. Another study showed that a greater number of infants demonstrated a >50% reduction in the daily crying time with the synbiotics.[125] Another recent study looked at the prebiotic effect of an infant formula supplemented with galacto-oligosaccharides in an RCT on colic symptoms. The authors noted that the prebiotic formula promoted the growth of *Bifidobacterium* and *Lactobacillus* while also inhibiting *Clostridium* growth and significantly lowered colic.[126] This suggests that probiotics, particularly including *L. reuteri*, could represent an important emerging treatment for infantile colic.[127,128] Although this is promising data, further research is needed to fully understand the mechanisms involved before these treatments can be recommended on a widespread basis.

Childhood Diseases Associated with Perturbation in the Microbiome

In this section we will review perturbations in the microbiome associated with malnutrition, atopic diseases (ie, eczema, allergies, and asthma), gastrointestinal (GI) diseases (ie, IBD and diarrhea), as well as the emerging roles of microbiota disruption in type I and II diabetes, obesity, and autism spectrum disorder (ASD).

Malnutrition. Childhood malnutrition is a general term that refers to both over- and under-nutrition, which is caused by various factors including inadequate nutrition intake, many times leading to delayed growth, as well as vitamin and mineral deficiencies.[129] Malnutrition is also the leading cause of death worldwide in children under 5 years of age and is a global health concern.[130] While therapeutic foods have reduced mortality rates in children with severe acute malnutrition (SAM), incomplete growth remains a problem in this population.[131] Interestingly, a recent study showed abnormal gut redox metabolism and a depletion in obligate anaerobic bacteria in children diagnosed with SAM.[132]

Emerging research is showing that factors associated with the development of a healthy microbiome, such as clean water and breastfeeding, may be deficient in malnourished children in developing nations, suggesting a compromised microbiome in malnourished infants in these countries.[130] In fact, *Bifidobacteria,* which is known to be an early predominant species in the healthy microbiome, has been shown to be lacking in the microbiome of malnourished children. The lack of this critical family of organisms may be a key player in the initiation of intestinal dysbiosis that gives rise to malnourished phenotypes. This finding may suggest that a putative protective mechanism could be put in place to ward off the onset of malnutrition, potentially by treating pregnant women.[131,133] Other strategies aimed at decreasing malnutrition in the at-risk populations include supplementation with probiotic yogurt,[68] sialyated milk oligosaccharides,[134] and other microbiota-derived foods.[52]

Atopic disease. *Atopic dermatitis (eczema).* Atopic dermatitis (AD), also known as atopic eczema, is currently estimated to affect approximately 2 million children worldwide with a lifetime prevalence of up to 20% and seems to be on the rise in post-industrialized nations.[135] This insidious immunological disorder is believed to be a product of genetic susceptibility interacting with environmental triggering events or exposures during critical developmental time windows. The multifactorial pathogenesis risk factors and/or contributing factors consist of polymorphisms in the filaggrin (filament aggregating protein) gene[136] in approximately 42%[135] of individuals, leading to excess *Staphylococcus aureus* and a dysbiotic skin microbiome. In addition, altered barrier function of the epidermis can lead to altered immune system regulation. Additional contributing factors include an altered enteric microbiome,[137] which leads to altered immune signaling.

There are two primary theories on the origins of AD: The "inside out" model gives credence to the enteric microbiome being disturbed. In this model, imbalances in the enteric microbiome give rise to inflammatory processes.[138–141] In support of this theory, aberrant microbiota profiles have been associated with AD. Song et al.[142] showed that enrichment of *Faecalibacterium prausnitzii* is strongly associated with AD. The authors recruited 90 AD participants to take part in a study to assess the microbiota profile in this population, and 42 controls. The authors noted an enrichment of *F. prausnitzii*, which they believed downregulated producers of SCFAs, exacerbated inflammatory cascades, and altered Th2-type immune responses. Other studies have shown altered gut microbiora profiles throughout the first year of life in IgE-associated AD and that the reduced abundance of immune-modulatory bacterial populations correlated with exaggerated inflammatory cytokine responses to Toll-like receptor (TLR) ligands.[143] Another study has shown that high fecal calprotectin levels at 2 months is an increased risk of developing AD and asthma/asthmatic bronchitis by the age of 6 years. The high levels of calprotectin correlated negatively with *Escherichia coli*. The authors hypothesize that this could explain the intestinal inflammation and subsequent development of AD and asthma via TLR-4 signaling mechanisms.[144]

In a small study of Chinese infants (15 cases, 10 controls) Tang et al.[145] found that that the relative abundance of *Bifidobacterium* and *E. coli* was decreased in eczema while that of *Klebsiella* and *Bacteroides* was elevated. The authors caution that interethnic variation should be a strong consideration in any microbiome study and that a low abundance of bacteria, and not a lack of microbial diversity, was a primary finding in their small study.

Contrary to the "inside out" hypothesis of AD is the "outside in" hypothesis, which suggests that the disrupted skin microbiome is the primary triggering event for AD. While the exact causal mechanisms remain to be seen, both hypotheses, which are not necessarily mutually exclusive, suggest that dysbiosis of the microbiome is a critical player that may be driving the disease processes and could be amenable to therapeutic intervention and that hygiene measures may be important to consider as well.[146]

Not only has microbiome modulation been investigated as a potential therapeutic strategy for improving AD symptoms and prophylaxis, but preventative strategies targeting aberrant maternal microbiomes during pregnancy and early postnatal administration of probiotics, prebiotics, and/or synbiotics have also been investigated. Multiple systematic reviews and/or meta-analyses have demonstrated the beneficial effects of probiotics and/or synbiotics in the prenatal and/or postnatal period in decreasing AD risks and as a possible prevention strategy.[147–153] A Cochrane review of prebiotics found that prebiotics added to infant formula reduced AD risk but was not effective in preventing other allergic diseases,[154] although the evidence was limited and caution was noted. Another recent meta-analysis, which examined both probiotic and prebiotics, found that prebiotics alone were not protective, although synbiotics (combined probiotics and prebiotics) did show a protective effect.[155] This latter meta-analysis also concluded that only probiotics containing the combination of non-spore *Lactobacilli* and *Bifidobacteria* reduced the incidence of AD. Further, a systematic review of RCTs with at least 4 weeks of treatment duration also found that supplementation with probiotics during pregnancy, breastfeeding, or early infancy reduced the risk of AD but no other allergic diseases.[156]

While these data are exciting and point to the possibility of treatment and/or prevention of AD via modulation of the microbiome, further research is needed to better determine treatment selections, formulations, dosing, timing, and other covariate environmental factors that may contribute to a treatment response (eg, breastfeeding, abstention from cigarette smoking, pets in the household, human migration)[157–159] need to be considered and researched before clinical treatment recommendations can be widely utilized, as there are still many unknowns and mixed results.

Allergies. Much like AD, allergies continue to grow in prevalence in developed nations, as well as in those who migrate to

developed nations, and are believed to be related to industrial practices related to biome depletion or exposure to a novel environment with novel regional gut microflora.[160,161] Risk factors associated with increased incidence of allergic disease include the maternal microbiome during pregnancy, mode of delivery, breast feeding versus formula feeding (especially during the first year of life), early or repeated exposure to antibiotics, introduction of solid food, and other environmental factors (eg, having older siblings and lack of pet exposure), which can negatively influence the development of the microbiome.

Animal models have shown that germ-free mice develop exaggerated airway inflammation and elevated levels of total serum IgE.[162–164] Also, antibiotic exposure in mice leads to a marked reduction in intestinal microbiota and increased serum IgE levels, which correlated with exaggerated allergic inflammation.[165]

Studies in humans have also found significantly decreased species diversity in those that go on to develop allergy, and strategies to try and prevent allergy development would need to be aimed at improving species diversity of the microbiome.[166,167]

Since colonization with a diverse microbiota early in life is critical for proper regulation and development of the immune system and since failure to do so can lead to a dysregulated immune system and increased incidence of allergic disease and other immunological disorders, therapeutic strategies aimed at primary prevention and treatment have been investigated that target the microbiome.[168–171] Most probiotic studies have included the bacterial strains *Bifidobacteria* and *Lactobacillus* and have primarily looked at prevention by administering the probiotics to pregnant women, breastfeeding women, or infants early during life.[156,172] In fact, the World Allergy Organization (WAO) has published recent guidelines based on the results of systematic reviews of probiotic and prebiotic interventions for the prevention and management of allergies.[173] While positive results have been demonstrated in AD, evidence is still lacking or conflicting in other allergic diseases, and other organizations have not taken a stance on the recommendations to support the usage of probiotics in pregnant women, breastfeeding mothers, or infants.[174,175] The reasons for the uncertainty are multifold. First, methodological constraints across studies limit comparisons, as the heterogeneity of study design, probiotic strains, dose, and timing are all factors that limit the generalizable nature of the findings and can lead to conflicting results.[150,172,176–182] The strongest evidence – although the best combination of strains and dosing is uncertain – supports the use of prebiotics and probiotics during the prenatal and early postnatal stages, beginning as early as day 1, with the most critical time quite possibly being the first month post delivery.[183] A recent systematic review and meta-analysis further validated this approach.[184] While evidence is currently lacking for allergic disease prevention or treatment, outside of AD prenatal and postnatal probiotic supplementation can be recommended for mothers and infants at high risk for allergic disease, per the WAO published guidelines.

Asthma. Asthma is the most prevalent chronic disease condition in children and is currently estimated to affect more than 300 million people worldwide.[12]

Recent evidence has pointed toward the role of early life respiratory infections, namely respiratory syncytial virus and human rhinovirus infections, as antecedents to asthma development.[185] It is believed that these early viral infections may alter the airway microbiome and further alter immune programming and signaling from the enteric microbiome.[186,187] Further, early microbiota disturbances with either lower microbial diversity or early colonization with opportunistic species have been associated with increased asthma risk.[188,189] Thus, this may be a possible explanation why probiotics and/or prebiotics may be ineffective in preventing asthma.[172,178]

Arrieta et al.[12] found that children at risk of developing asthma exhibited transient gut microbial dysbiosis during the first 100 days of life. The lack of species diversity was primarily due to a loss of *Lachnospira, Veillonella, Faecalibacterium,* and *Rothia*. The reduction in bacterial species correlated with reduced levels of fecal acetate and disruption of enterohepatic metabolites. The authors then demonstrated a causal role of these bacteria in asthma by demonstrating abrupt amelioration of airway inflammation when these missing taxa were inoculated into germ-free mice. Interestingly, a recent study showed that the Amish environment provides protection against asthma by shaping the innate immune system via exposure to microbial populations that are lacking in industrialized societies.[190]

A recent systematic review found no effect of probiotics in the prevention of allergic disease or asthmatic conditions, although there has been low-quality evidence that pregnant women or breastfeeding mothers who take probiotics or give them to their infants reduced the risk of AD in their children.[156] Thus, there is not sufficient evidence to recommend probiotics for the primary prevention or treatment of asthma-related disorders,[191] but there are many methodological issues that impact the generalizability of these findings. Indeed, further research is needed to delineate the possible prevention and/or treatment strategies related to the use of these compounds in combating pediatric asthma.

Gastrointestinal disease. *Inflammatory bowel disease.* IBD refers to relapsing inflammatory disorders of the GI tract. These disorders are primarily a result of genetic, immunologic, microbial, and environmental (eg, diet) factors that converge together.[192] The two conditions that represent the clinical phenotypes of IBD consist of ulcerative colitis (UC) and Crohn's disease (CD). Gut dysbiosis is thought to contribute to the length, severity, and chronic nature of intestinal inflammation in IBD. It is of note that no genetic etiology could be found in 77% of patients with CD and in 80% of patients with UC.[192,193] In addition, twin studies discordant for UC suggest that alterations in the enteric microbiome precede the development of disease.[194] Interestingly, even single nucleotide polymorphisms (SNPs) associated with IBD have identified

genes that are involved in pathways that modulate host response to microbial stimuli.[195,196] These findings have led to the speculation that IBD disease processes may be more of a result of environmental influences interacting with predisposing genetic factors to give rise to the etiological basis of the diseases. Further, this provides compelling evidence for host–microbe interactions as being a central component to the development of IBD. Indeed, groups have hypothesized that the rise in IBD is a result of modern practices such as increased C-sections, sanitization practices, dietary changes, antibiotic overusage, alterations in *E. coli* and *F. nucleatum*, and other factors associated with biome depletion.[197–202]

Studies on pediatric irritable bowel syndrome (IBS) and the associated microbiome abnormalities are limited at this time. Saulnier et al.[203] found significantly greater *Proteobacteria*, with a prominent component of the *Proteobacteria* being *Haemophilus parainfluenzae* in pediatric patients with IBS. Interestingly, subtypes were able to be identified with 98.5% success rate using a limited set of bacterial species. Further, a novel *Ruminococcus*-like bacterium was associated with IBS, and the frequency of pain correlated with an increased abundance of several *Alistipes* taxa. Other studies have also shown microbiota alterations in pediatric IBS.[204]

Therapeutic strategies aimed at targeting the microbiome for IBD have included dietary changes, such as elimination of Westernized dietary practices. Such diets, which are low in fiber but high in fat and protein, are thought to be responsible for decreased microbial diversity and reduced SCFA production.[205,206] Interestingly, treatment response to steroids is correlated with a more diverse microbiome in patients treated for UC.[207] Other therapeutic options targeting the microbiome have included probiotics,[208–212] antibiotics,[211,213–218] fecal microbiota transplantations (FMTs),[219–223] and the recently defined colonies of human anaerobic intestinal microbiota,[224] although data in pediatrics is limited to date, with many of these therapies being tested primarily in adult populations. It should be noted that no trial to date has demonstrated a beneficial effect of probiotics in the treatment of CD.[225,226] It should be noted that in the work of Bousvaros et al.[226], the probiotic and placebo both contained inulin, with a higher inulin dose in the placebo group, which might have confounded the results, although the dose of the inulin was small. Evidence for probiotics in UC is more promising,[227,228] but there have been no studies to date on prebiotics in children.[229] Further, to date, these therapeutic techniques have demonstrated varying levels of success, with no single treatment modality demonstrating to be truly restorative and/or curative.[230] While the future looks encouraging for investigating potential treatment and preventative strategies related to enteric microbiome disturbances in IBD, further research with long-term follow-up is needed to delineate the best approaches and practices and for whom these approaches may benefit the most.

Antibiotic-associated diarrhea. Antibiotic associated diarrhea (AAD) is a common adverse effect of broad-spectrum antibiotic medications, especially those (eg, clindamycin, penicillin, etc.) that target anaerobic bacterial populations,[231,232] and is related to the disruption of the enteric microbiome.[233] Interestingly, two recent Cochrane reviews demonstrated that probiotics administration prior to antibiotic treatment has the ability to prevent AAD presumably by mitigating or offsetting some of the damaging effects on the microbiome caused by antibiotics.[232,234] Furthermore, the evidence was strongest for high-dose probiotics at >5 billion CFUs/day with a number needed to treat (NNT) of 7 (ie, the number of patients needed to be treated in order to get a treatment response in at least one patient is 7) in providing benefits against AAD as of 2011, although this was updated in 2015 to an NNT of 10 with recommendations for *Lactobacillus rhamnosus* or *S. boulardii* at 5 to 40 billion CFUs/day. However, the authors caution that larger trials are needed and that it is too premature to draw conclusions related to efficacy and safety, and that future trials need to incorporate standard and valid outcome measures to further evaluate probiotics in AAD.

Another concerning adverse effect pertaining to AAD is the overgrowth of *C. difficile*.[235] Probiotics have been shown to be effective at preventing *C. diff* infections associated with antibiotic usage,[236–238] with probiotics helping children with a reduced risk of acute diarrhea by 57% compared to 26% in adults.[237] Lau and Chamberlain showed that the pediatric use of probiotics prevents antibiotic-associated *C. diff* infections by 66%.[239] The best evidence is for probiotics co-administered with antibiotics.[232,240]

Diabetes. In this section we will cover the emerging role of microbiota disruption in diabetes. Evidence from preclinical and clinical populations will be reviewed, as well as the potential roles of antibiotic exposures during critical time windows being a possible etiological factor involved in the origin of diabetes, and the roles that probiotics and other therapeutic techniques aimed at modulation of the enteric microbiome or their metabolic end products may have in the treatment and/or prevention of diabetes.

Type I diabetes. Type I diabetes mellitus (T1DM) is primarily caused by genetic vulnerability to pancreatic β-cell destruction through autoimmune processes, which leads to a lack of insulin and elevated blood glucose.[241,242] While there is a strong genetic contribution to T1DM, to date the genetic basis of TIDM appears incomplete and has not fully explained the increasing prevalence of the disorder.[243–245] Research has since started to focus on gene–environment interactions that may increase risk for T1DM that go beyond genetic factors alone, and focus on genetic susceptibility interacting with environmental factors at critical time points during development. Interestingly, environmental factors that have been linked to increased risk for T1DM in genetically susceptible individuals include factors associated with the development and maintenance of the microbiome, which can affect immune system signaling and programming (eg, C-sections vs. vaginal

birth, breast feeding vs. formula feeding, viral infections, and antibiotic exposures).[246,247]

Indeed, abnormalities in gut permeability and the microbiome have been linked to T1DM,[248] although the data is mixed[249] and may be related to the presence of autoantibodies[250,251] and/or the age or time between seroconversion and diagnosis.[252] Animal studies have implicated the *Bacteroidetes:Firmicutes* ratio at or before the onset of the disease as well as the levels of *Bifidobacterium* and *Lactobacillus* at the later stages of disease progression. It is believed that these imbalances may alter SCFA production, especially butyrate, and alter microbiome diversity and intestinal permeability, which may precede the onset of the disorder.[253–255] These findings have been corroborated in human studies.[248,256] The lack of the SCFA butyrate has also been hypothesized to lead to decreased mucin production, decreased tight junctions assembly, and decreased epithelial cell integrity.[254] A noteworthy recent study of the enteric microbiome in infants with the highest genetic predisposition to T1DM showed a 25% reduction in microbial diversity in those who developed the disease and that the shift in microbial composition of the microbiome occurred prior to disease onset but after seroconversion and correlated with disease progression.[252]

Since microbiome changes precede the onset of T1DM, microbiome-targeted intervention strategies may be able to halt disease progression or possibly lessen morbidity. In fact, a recent study investigated the use of probiotics in infants to prevent the development of T1DM.[257] The results indicated that infants who were provided probiotic supplementation days 0–27 after birth was associated with a 60% decreased risk of islet autoimmunity when compared to supplementation after 27 days of birth or no probiotics. While this study needs to be replicated and extended in additional research studies, it underscores the importance of the development of the microbiome and potential treatment strategies aimed at targeting and improving the gut microbiome milieu in the hope of preventing the development of T1DM and raises questions regarding other treatment modalities, namely FMT, prebiotics, synbiotics, and their roles in treatment of T1DM. Since much of the evidence is based on animal models, the time is ripe for further human clinical trials to further our understanding of which probiotics are most beneficial, optimal dosing, timing, and other important clinical implications pertaining to prevention strategies and possible therapeutic interventions pertaining to T1DM.

Obesity and type II diabetes. Obesity is a complex disease process that can increase the risk of type II diabetes mellitus (T2DM). Emerging research is showing that obesity is much more than the "calories in, calories out" hypothesis and that factors beyond sedentary lifestyle, exercise, diet, and genetics seem to be at play, with increased recognition that the enteric microbiome may underlie inter- and intra-subject variation in relation to weight loss as well as the development and maintenance of obesity.[258] Interestingly, agricultural practice has known for many years that providing subtherapeutic doses of antibiotic medications to livestock early in life is an efficient method of enhancing the animals' growth, and the earlier the introduction of the antibiotics, the more profound the effects in the animals.[259,260] The presumed method of this enhanced growth of the animals is through alteration of the hosts' microbiota and altering their metabolic function. This knowledge has prompted an investigation to see whether early life antibiotic exposures lead to obese outcomes in children.[260] The timing of antibiotic exposures early in life for infants appears to be just as critical as the timing of "fattening up" livestock for the harvest. A study by Trasande et al showed that antibiotic exposure in the first 6 months of life is associated with consistent increases in body mass from 10 to 38 months even when controlling for other important social and behavioral risk factors.[261,262]

The microbiota signature of obesity and the subsequent T2DM appears to mirror that of T1DM, as there is a relative reduction of *Bacteroidetes* with less bacterial diversity.[262,263] Further, this absence of bacterial diversity and lack of gene richness is associated with higher adiposity, dyslipidemia, impaired glucose, and low-grade inflammation.[264,265] Low-grade inflammation is associated with circulating levels of LPS, which may be the molecular link between a high fat diet and insulin resistance.[90,266,267] It is of note that, while there are putative factors that have been associated with altered microbiota and subsequent development of obesity and/or T2DM, little is known about the microbial characterizations that precede disease onset and the relation between disease pathogenesis and microbiota disturbance in pediatric populations. A recent study by Riva et al.[268] demonstrated an altered microbiota in obese children compared to normal-weight children. Obese children had increased correlation density and clustering of operational taxonomic units. *Bacteroidetes* was a predictor of BMI z-scores. The *Firmicutes:Bacteroidetes* ratio was elevated, which has been replicated in other studies.[269] Further, SCFAs were higher in obese children, a finding that has also been shown in obese adolescents.[270–272] Elevations in *Enterobacteriaceae* and lower abundance of *Akkermansia muciniphila*-like bacteria have been found in obese preschool children.[273]

While almost all animal studies have shown the anti-obesity property of probiotics, the mechanisms of action seem to be related to anti-inflammatory and/or improving oxidative stress and modulating energy homeostasis.[274] A study on perinatal probiotic usage to assess childhood growth patterns and the development of overweight phenotypes showed that administration of *Lactobacillus rhamanosus GG* treatment from 4 weeks before birth in the mother through the first 6 months of life in the child significantly attenuated excessive weight gain during the first year of life with the peak effect at 4 years of life but a diminishing effect after this peak.[275] A pediatric study of probiotics for obesity-related non-alcoholic fatty liver disease showed a significant decrease in aminotransferase activity, although other clinical endpoints were not significant,

leading the authors to conclude that the use of *L. rhamanosus GG* should be considered in obese children noncompliant with lifestyle interventions.[276] An 8-week study found significant effects of synbiotics on cardiometabolic risk factors in obese children and adolescents.[277] However, a prebiotics-only study showed no effects in obese children and adolescents.[278]

While the contributions of enteric microbiome disruption in the etiology and/or pathophysiology of childhood obesity and subsequent development of T2DM is an emerging topic, further research is needed to better understand how microbiome disruptions early in life lead to the onset, development, and maintenance of these disorders and the optimal therapeutic strategies targeting the microbiome for treatment or prevention of disease.

Autism spectrum disorder. Emerging research is finding that the enteric microbiome and its metabolic byproducts, including PPA, play a major role in normal brain and behavioral development[279] and are altered in persons with ASD.[280–285] Interestingly, neurodevelopmental abnormalities, which include ASD features, are seen in individuals with impaired PPA metabolism.[27,286,287] PPA has been shown to be elevated in the stool from individuals with ASD[288] but not in every study.[289] Adams et al.[289] speculated that the lower incidence of SCFAs in the stool in their study could be consistent with the PPA theory, as this could potentially mean that more SCFAs are being absorbed and entering the blood stream and exacerbating ASD symptoms.

Hsiao et al.[290] conducted a landmark mouse study which demonstrated that a probiotic treatment could significantly attenuate ASD-like behaviors, thereby suggesting that enteric microbiota disruptions could potentiate ASD-like behavior. Interestingly, mice with ASD-like symptoms had markedly elevated levels of the phenolic derivative 4-ethylphenylsulfate at 46 times higher concentration than the control cohort mice. The chemical properties of 4-ethylphenylsulfate are structurally similar to those of p-cresol (4-methylphenol), which alters cellular membrane permeability, redox activity, and ion channels, and has been shown to be elevated in urine from individuals with ASD.[291–295]

Over the last 15 years, we have developed a PPA animal model of ASD.[16,17] In the initial model, brief intracerebroventricular PPA infusions into adult rodents produced reversible ASD-type behaviors such as reduced social interactions,[296] stereotyped behavior,[297] tics,[297] hyperactivity,[297,298] and cognitive and sensorimotor deficits,[299] as well as ASD-associated biological abnormalities such as reactive astrocytosis and activated microglia,[296,297,299] abnormalities in redox, lipid, phosphatidylethanolamine, mitochondrial, acyl-carnitine, and carnitine metabolism,[29,297,298,300] and electrographic abnormalities in the hippocampus, neocortex, and basal ganglia.[297]

The PPA theory of ASD[24,84,298] suggests that ASD may be a result of disturbances in the enteric microbiome resulting in the production of elevated levels of PPA in genetically susceptible individuals during a critical neurodevelopmental period. Microbes that produce PPA, including *Clostridia*, *Bacteroides*, and *Desulfovibrio* species, are reported to be in abundance in ASD patient cohorts.[301] PPA as well as other SCFAs can alter diverse metabolic and immune pathways, gene expression, and synaptic plasticity in a manner that is consistent with findings of ASD. The PPA theory of ASD may also offer a potential explanation of why certain diet manipulations may provide therapeutic benefit for certain children with ASD, as modifying the gut ecosystem through dietary changes may influence the taxa represented in the microbiome and the SCFAs they produce.

Studies that have examined the enteric microbiome in children with ASD have shown decreased species diversity and overrepresentation of certain species such as *C. difficile*.[302,303] Studies have suggested that children with regressive-type ASD may have particular abnormalities in their gut microbiome,[304] and that children with alterations in particular bacterial species may constitute a subgroup of children with ASD who present with GI symptoms at the time of or prior to the onset of ASD symptoms, suggesting that imbalances in the microbiome may be part of the underlying ASD etiology.[305]

Small treatment trials associated with manipulating the microbiome in ASD have been conducted, and further research into this approach has been suggested.[2]

The antibiotic vancomycin broadly targets Gram-positive bacteria, including the anaerobic bacteria *Clostridium* genus, and is believed to have a favorable safety profile when administered, orally since under normal physiological circumstances vancomycin is not absorbed from the GI tract into the circulation.[306,307] Through targeting bacteria that produce PPA, vancomycin has been shown to decrease PPA production.[308]

In a small, partially blinded, 8-week clinical trial conducted in the United States on children with ASD, oral vancomycin significantly improved GI symptoms and irritability,[305] supporting the PPA rodent model of ASD.[17] Because of the potential to develop vancomycin resistance enterococcus, this evidence provides more theoretic support for the PPA model of ASD rather than direct practical therapeutic implications.

To date, there have been several therapeutic trials investigating the efficacy of probiotics for the treatment of ASD symptomatology.[289,309–313]

Adams et al.[289] found that in children with ASD, probiotics lowered stool SCFAs (ie, acetate, butyrate, propionate, valerate) but did not significantly change bacterial concentrations other than marginally resulting in higher lactobacillus concentrations. Behavioral and/or GI symptoms or effects of probiotics were not discussed in the study.

An open-label trial found that twice-daily treatment with *L. acidophilus* (5×10^9 CFU/g) for 2 months decreased the levels of D-arabinitol, a metabolite of *Candida* species, and led to a significant improvement in the ability of the children to concentrate and carry out orders, although there is no mention of how this was assessed.[309]

Parracho et al.[310] conducted a 12-week, double-blind, placebo-controlled crossover trial of *Lactobacillus plantarum WCSF1*. The study was limited by a high dropout rate, primarily in the baseline period, and high inter-individual variability. Of the 62 children aged 3–16 years that enrolled, 17 completed the trial. The authors strongly advised the utilization of subgroups of children with ASD in future trial designs. However, the probiotic therapy did increase *Lactobacilli* and *Enterococci* bacterial species and decrease *Clostridial* species in comparison and significantly affected stool consistency. Greater improvements in total and subscale scores on the Developmental Behavior Checklist were found in the probiotic group.

West et al.[311] conducted an uncontrolled survey study of caregivers of children with ASD that had GI distress and received the probiotic Delpro®. Delpro® contains five probiotic strains formulated with the immunomodulator Del-Immune V®. Caretakers assessed ASD symptoms before and after 21 days of treatment with Delpro® using the Autism Treatment Evaluation Checklist. Of note, 48% of caregivers reported decreases in diarrhea severity, and 52% reported decreases in constipation severity. In addition to improved GI symptoms, 88% of caregivers endorsed decreases in ASD symptoms. These data will need to be considered in the context of this being an uncontrolled trial with no placebo, blinding, or randomization.

Another study showed that the probiotic Children Dophilus, which contains two strains of *Lactobacillus* (60%), 2 strains of *Bifidumbacteria* (25%), and one strain of *Streptococcus* (15%), given three times daily normalized the *Bacteroidetes:Firmicutes* ratio and the concentration of *Desulfovibrio* spp. and *Bifidobacterium* spp. in the stool of Slovakian ASD children.[313] The authors did not remark on the effects of probiotic therapy on ASD symptoms.

A case report[312] documented improvement in behavior of a child with ASD with probiotic therapy. The reported improvements were based on school reports and the child expanding the variety of foods eaten. Withdrawal of the probiotic therapy was reported to result in a regression to the child's baseline within 4 days, which was reversed when the probiotic was reintroduced.

In a survey of physicians regarding alternative medicine for treatments of children with ASD, 60% of physicians endorsed the usage of probiotics given the high incidence of GI disturbance and the favorable safety profiles of probiotics.[314]

While preliminary studies suggests that probiotics may improve microbiome abnormalities in children with ASD and limited data suggest that behavior may also be improved with probiotics, the methodological limitations of the studies conducted to date and the lack of knowledge of the long-term effects of probiotic therapy limit the ability of treatment recommendations for children with ASD.

Individuals with ASD are different from many other pediatric populations due to their deficits in communication and language. In many circumstances, signs of GI disturbances may not be obvious and may manifest primarily by behavioral changes.[315] For example, abdominal pain, gastroesophageal reflux disease, and/or constipation can manifest as vocal symptoms such as frequent repetitive throat clearing or swallowing and/or screaming, motor behaviors such as facial grimacing, teeth grinding, chewing on clothes, applying pressure to the abdomen, or aggressive or self-injurious behavior; and/or general behaviors such as sleep disturbance or irritability.[315] Thus, in order to consider the most appropriate study design for investigating enteric microbiome treatments for children with ASD, we held a Microbiome Workshop at Arkansas Children's Research Institute as an extension of the 1st International Symposium on the Microbiome in Health and Disease with a Special Focus on Autism (www.microbiome-autism.com). The working group included clinicians, research scientists, and parents of children with ASD, and a summary of the meeting was published.[2]

Conclusions: Promise, Cautions, and Future Directions

The relationship between enteric microbiome and childhood disease is an area that is flourishing with findings that may greatly impact the understanding of pediatric health and the consequences of certain iatrogenic practices that may impact a child's lifelong risks for disease. These findings may lead to new directives and guidelines related to the risk–benefit ratios of many treatments, including prenatal and early life antibiotic exposure, maternal heath, prenatal, perinatal, and neonatal care practices, hospitalization, human migration, and diet. Collectively, they may give rise to new drug discovery, development, and utilization aimed at modulating the microbiome to ward off diseases associated with microbiome disruption.

The use of probiotic supplementation during pregnancy and early infancy seems a promising area for primary prevention and treatment, and, at this stage, appears safe. In the future, it may be possible to utilize probiotics to prevent infections so that traditional treatments such as antibiotic therapy, which can lead to microbial resistance, is minimized. However, further research is needed to understand the optimal probiotic composition as well as the frequency, duration, and timing of dosing. It should be noted that this area of research, while promising, is far from being integrated into systematic guidelines for pediatric medicine. There is a need for standardization of the identity and composition of probiotics, and even screening for the presence of toxic contaminants, which can be extremely variable from product to product.[316,317]

Fecal microbial transplantation in certain conditions, such as refractory *C. difficile* colitis, has shown remarkable safety and efficacy in adult patients, but studies in children and for other diseases are limited for this treatment. The limited data on fecal microbial transplantation in pediatric populations, so far, seems to demonstrate similar efficacy, although long-term safety studies are needed.[318–321] The critical need for a suitable donor, a "stable" microbial inoculum, and the potential for transfer of infectious agents or antibiotic resistance makes the emerging research of anaerobically

cultured human microbial communities an interesting and rational alternative.

Furthermore, although properly designed trials in defined diseases are going on, there is little information on the long-term effects of treatments that influence the microbiome. It could be that the treatment of one disease may increase the risk for another at a later time. For example, antimicrobial treatment of *Helicobacter pylori* reduced the incidence of peptic and duodenal ulcers but increased the incidence of esophageal cancer.[322] This issue is particularly important in the use of prophylactic treatments, such as probiotics, for the prevention of childhood diseases in healthy children or those with minor afflictions (colic, diarrhea) since they are incapable of informed consent. There is a need for translational animal models to understand the mechanisms by which the microbiome modulates human disease so that the most optimal manner for manipulating the microbiome is determined in preclinical studies.

It appears that the microbiome may be disrupted early in life as an unintended consequence of some essential and life-saving early life treatments such as C-sections and antibiotics use, leading to increased risk for obesity and immune and neurodevelopmental conditions[46] particularly in developing nations.[50] With this new knowledge, it may be possible to make the long-term consequences of these essential medical treatments safer by restoring the microbiome through probiotics or human microbial transfer. Thus, there is cautious optimism of this emerging field in pediatric health and disease.

Author Contributions

JS, DM, and REF contributed to the writing of the manuscript and all aided in the compilation and conclusions for each section. All authors reviewed and approved the final manuscript.

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