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## **The microbiome and paediatric gut diseases**

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## **WHAT IS KNOWN ABOUT THE TOPIC**

- The human gut microbiome plays a crucial role in whole-body health.
- The gut microbiome has been implicated in the underlying pathogenesis of non-infectious gut diseases.

## **WHAT THIS STUDY ADDS**

- The gut microbiome is altered in paediatric gut disease but its exact role in primary disease pathogenesis is still unknown.
- There are currently very few implications for clinical practice from the role of gut microbiome in paediatric gut disease.
- In the future, the gut microbiome may aid in disease differential diagnosis, prediction of clinical outcomes and comprise a target for therapeutic interventions.

## **ABSTRACT**

In the human gut resides a vast community of microorganisms which perform critical functions for the maintenance of whole-body homeostasis. Changes in the composition and function of this community, termed microbiome, is believed to provoke disease onset, including non-communicable diseases. In this review, we debate the current evidence on the role of the gut microbiome in the pathogenesis, outcomes, and management of paediatric gut disease. We conclude that even though the gut microbiome is altered in paediatric inflammatory bowel disease, coeliac disease, intestinal failure, necrotising enterocolitis and irritable bowel syndrome there are currently very few implications for unravelling disease pathogenesis or guiding clinical practice. In the future, the gut microbiome may aid in disease differential diagnosis, prediction of clinical outcomes and comprise a target for therapeutic interventions.

## INTRODUCTION

The human gastrointestinal tract harbours a diverse community, termed as microbiome. This microbial community possesses genes for metabolic pathways producing thousands of metabolites, the majority of which our body is unable to encode. Gut microbes produce vitamins for the host and maintain trophic and immunoregulatory functions; the effects of which extend beyond the gut. Our interest in the role of gut microbiome in health and disease has expanded further recently with the advent of high-throughput sequencing and bioinformatic tools to process the vast amount of data generated. Here, we summarise the current evidence which implicates gut microbiome in the onset, management and disease outcomes of non-communicable conditions of the gut in children (Figure 1) and opportunities for microbial therapeutic applications (Figure 2).

### **Preterm infants and necrotizing enterocolitis**

The gut microbiome develops from birth and is influenced by a range of maternal, clinical, and environmental factors such as birth mode and receipt of breast milk.<sup>1</sup> As a prime example lactate and acetate are the predominant short chain fatty acids (SCFA) in infants receiving human milk whereas formula-fed infants have less lactate and higher concentrations of propionate and butyrate.<sup>2</sup> The measurable impacts of such factors on the microbiome diminish over the first 18 months of life, and by month 30 the microbiome is highly individual and relatively stable.<sup>1</sup> The tangible ability to modulate the gut microbiome over early life has been described as ‘the window of opportunity’ for promoting short- and long-term health.

The single most important factor influencing the development of the gut microbiome over the first year of life is the receipt of breast milk, which contains a wide range of prebiotic and antimicrobial compounds. Of particular interest are human milk oligosaccharides (HMOs), a family of structurally diverse, complex unconjugated sugars, that are absent from standard preterm formula milk. HMOs cannot be digested by the infant, so they reach the lower gastrointestinal tract intact where they act as growth substrates for specific bacteria, most notably *Bifidobacterium* spp.<sup>3</sup> While primarily considered a prebiotic, increasing evidence also suggests HMOs may act directly on the human immune system.

Whilst the gut microbiome of term infants has been well described, the preterm infant gut microbiome is less well characterised. Nonetheless, evidence clearly shows that infants born significantly premature, who will initially be cared for in the neonatal intensive care unit (NICU), have altered microbiome development. The diversity of the preterm microbiome is low and six bacterial genera in *Staphylococcus*, *Enterococcus*, *Klebsiella*, *Enterobacter*, *Escherichia*, and *Bifidobacterium* are usually abundant in the preterm gut.<sup>4</sup> Notably, all but *Bifidobacterium*, are widely considered as pathobionts.

The gut microbiome has been studied in relation to necrotising enterocolitis (NEC), a leading cause of mortality in the most premature infants. Typically, NEC infants have higher abundance of Proteobacteria, lower *Bifidobacterium*, and reduced stability of the microbiome before disease onset, in comparison to matched controls.<sup>4 5</sup> While not possible to separate cause from effect in such observational studies, the reported associations suggest that a stable gut microbiome with higher *Bifidobacterium* may reduce the risk of NEC.

Separately, human and animal work has shown that a specific HMO, disialyllacto-n-tetraose (DLSNT), is higher in healthy compared to NEC infants and can reduce the disease in a neonatal rat model.<sup>6 7</sup> More recently, these results were validated in a large independent cohort of preterm infants, where DLSNT alone could predict the risk of NEC with 90% sensitivity/specificity.<sup>8</sup> This study further performed metagenomic sequencing of longitudinal stool samples and showed the gut microbiome of preterm infants receiving high levels of DLSNT in mothers' milk was more likely to transition into a *Bifidobacterium* rich gut microbiome.

The use of probiotics has been gaining interest considering studies supporting higher *Bifidobacterium* levels are associated with reduced disease. Although recent meta-analyses show that overall probiotics significantly reduce NEC and The European Society of Paediatric Gastroenterology, Hepatology and Nutrition provide conditional recommendation for their use,<sup>9</sup> it is critical to determine the optimal bacterial strain(s), dosages, and their growth substrates. Equally, prebiotic supplements including galacto- and fructo-oligosaccharides and HMOs are being exploited by infant formula industry to replace or restore absent human milk components. It is too early to know if such prebiotic supplements can reduce disease risk, but early evidence supports they can increase the abundance of potentially beneficial bacteria, such as *Bifidobacterium*.<sup>10</sup> The engineering of formula milk should be done with caution and

using evidence to inform what components to add. For instance, 2'Fucosyllactose is primarily used owing to its abundance and lower cost to manufacture, but HMOs such as DSLNT may be more beneficial. There is also a risk that specific HMOs may have negative impacts in certain settings,<sup>11</sup> and that supplemented formula may discourage mothers from expressing breast milk.<sup>12</sup>

With prebiotics and probiotics providing exciting targets for novel therapeutic intervention in early life, it is important that strong evidence underpins the specific selection, which is unlikely to be the same for all infants.

### **Intestinal failure**

Intestinal failure (IF) is the critical reduction of functional gut mass below the minimum needed to absorb nutrients and fluids, such that parenteral nutrition (PN) is required. The intestine is either too short, in short bowel syndrome due to surgery or congenital conditions, or dysfunctional.

Previous studies profiling the microbiome in children with IF (4 months to 17 years), found an overall reduction in bacterial diversity,<sup>13</sup> with a striking dominance of Proteobacteria, mainly members of *Enterobacteriaceae*,<sup>13-15</sup> which in healthy humans represent 1-2% of gut microbiome. Overabundance of Proteobacteria in IF is most likely explained by the lack of dietary fermentable substrate essential for bacterial growth. This 'gut starvation' effect and lack of inter-species competition and cross-feeding could offer an advantage for subdominant species to increase over the normal gut habitants. Patients with IF also present depletion of Bacteroidetes,<sup>15</sup> and minimal levels of Firmicutes,<sup>13 14</sup> although abundance of *Lactobacillus* can be particularly high in patients with IF,<sup>13-15</sup> with occurrence of uncommon species like *Lactobacillus mucosae*.<sup>13</sup>

Beyond the characterization of the gut microbiome in IF, a few studies have explored relationships with disease outcomes and most existing research is of cross-sectional design. A lower microbial diversity and overgrowth of Proteobacteria were associated with the development of IF-associated liver disease.<sup>14 16</sup> A possible mechanism by which Proteobacteria could lead to liver injury is by excessive amounts of pro-inflammatory

lipopolysaccharide.<sup>17</sup> *Lactobacillus* might also induce liver damage via increased bile acid deconjugation.<sup>14</sup> Small intestinal bacterial overgrowth is common in patients with IF and has been associated with PN dependence.<sup>18</sup> Overgrowth of gram-positive anaerobes in the colon such as *Lactobacillus*, as well as poor metabolism of bacterial produced D-lactic acid, and transfer in circulation, could lead to D-lactic acidosis, which can cause neurological symptoms. In other studies, a low abundance of Proteobacteria, especially *Enterobacteriaceae*, and a high abundance of Firmicutes, especially *Clostridium* were related with positive nutritional outcomes.<sup>14 15 19</sup>

Data from the effect of interventions with dietary fiber, SCFA and probiotics on gut adaptation in patients with IF holds promise, but the evidence remains limited and conflicting.<sup>20</sup> A recent RCT showed that the use of *Lacticaseibacillus rhamnosus* and *Lactobacillus johnsonii* did not change major bacterial groups in patients with short bowel syndrome weaned from PN.<sup>21</sup> Antibiotics are frequently used to treat small intestinal bacterial overgrowth. However, using scheduled antibiotics in children with IF may lead to a higher abundance of gram-negative pathogens with potentially negative downstream consequences.<sup>19</sup> In paediatric IF, treatment with FMT has only been described in a single patient with short bowel syndrome and recurrent, debilitating D-lactic acidosis, which was successfully treated.<sup>22</sup>

Patients with successful intestinal adaptation can wean-off PN. Previous research showed that patients weaned-off PN had a higher microbial diversity than patients still on PN, but their diversity was still lower than healthy controls.<sup>13</sup> Prospective studies are required next to assess longitudinal changes from IF initiation and during the process of gut adaptation and associate these with clinical outcomes. There is also potential to use the gut microbiome as a biomarker to guide clinical practice and timing of transition from PN to enteral/oral nutrition.<sup>13</sup>

### **Inflammatory bowel disease**

Crohn's disease (CD) and ulcerative colitis (UC), collectively known as inflammatory bowel disease (IBD), are chronic, inflammatory conditions of the gastrointestinal tract. In IBD, the microbiome diversity and stability are reduced,<sup>23 24</sup> and bacterial composition is different to that of healthy controls; with these differences being more pronounced in CD than UC.<sup>25 26</sup>



The most consistent findings among studies include enrichment of Proteobacteria and decline of Firmicutes.<sup>26 27</sup> Compared to quiescent disease, patients with active IBD present a higher decrease in microbiome diversity,<sup>28</sup> and a lower abundance of *Clostridium coccoides*, *Clostridium leptum*, *Faecalibacterium prausnitzii* and *Bifidobacterium* spp.<sup>29</sup> *Escherichia coli* strains with adherent and invasive properties have also been implicated in IBD pathogenesis,<sup>30</sup> particularly in CD affecting the ileum where their frequency is high. The largest study profiling the microbiome in paediatric IBD [mean (SD) age in years: 12.4 (3)] reported an increase in abundance of *Neisseriaceae*, *Gemellaceae*, *Fusobacteriaceae*, *Veillonellaceae*, *Pasturellaceae*, *Enterobacteriaceae* and a decrease in abundance of *Bacteroidales*, *Clostridiales*, *Erysipelotrichaceae* and *Bifidobacteriaceae* in mucosal biopsies and faeces of new onset CD.<sup>31</sup> Nonetheless, it is still unclear whether microbiome shifts are causative to the IBD pathogenesis or rather an epiphenomenon of disease, its treatment and changes in dietary habits. There is scarce literature which has explored ethnic or geographical differences in the microbiome of children with IBD but the fact that consistent features of IBD microbial dysbiosis have been reported among studies performed in different countries suggest that any such differences are of small size or importance.

Metabolomic profiling provides information for the gut microbiome functionality. Compared with healthy controls, alterations in the concentration of faecal SCFA have been reported in IBD, but the direction of these effects is inconsistent in the literature.<sup>32 33</sup> Recent studies have also described distinctive faecal metabolomic signatures in paediatric CD and UC using untargeted metabolomic applications.<sup>34</sup>

There are few studies which aimed to modulate the gut microbiome of patients with IBD to influence disease outcomes. Following faecal microbiota transplantation (FMT), a cumulative remission rate of 51% and 36% was reported for patient with CD and UC, respectively.<sup>35</sup> Interestingly, endoscopic data showed promising results with the use of multiple FMT infusions in distal UC (endoscopic response/remission: 24-55% for FMT vs 5-17% for placebo). There are scarce endoscopic data in CD, with one study reporting no effect.<sup>35</sup> There are only six studies in paediatric UC, in a total of 34 patients, and only two paediatric studies in CD, in a total of 13 patients, reporting a cumulative remission rate of 54% and 23%, respectively. In adults, an increase in  $\alpha$ -diversity or microbial richness and a shift towards the donor profile was associated inconsistently with disease improvement. A variety of microorganisms were reported to be associated with therapeutic outcomes in adults with

IBD, following FMT.<sup>35</sup> Long-term follow-up data regarding FMT durability in IBD are lacking, particularly in children. From adult studies the available data suggest that disease relapse will invariably occur and some form of maintenance therapy or repeated course of FMT may be required.<sup>35</sup>

Probiotics, prebiotics and synbiotics have been studied as potential disease activity modifiers in IBD. Encouraging efficacy signals generated in animal experiments were not replicated in subsequent clinical research,<sup>36 37</sup> and with the exception of certain probiotic combinations in induction of remission of mild-to-moderate UC and prevention of pouchitis.<sup>38</sup> Data in children are scarce. An RCT in paediatric CD failed to demonstrate the effectiveness of *Lactobacillus GG* over placebo in prolonging duration of remission.<sup>39</sup> Two well designed paediatric studies in UC showed that the VSL#3 probiotic combination,<sup>40</sup> and rectal enemas of *Limosilactobacillus reuteri* ATCC 55730,<sup>41</sup> can improve induction and maintenance of remission, endoscopic and histological findings compared to placebo.

Antibiotics can be efficacious in certain IBD phenotypes, including pouchitis and perianal fistulising CD. In children with mild-to-moderate CD metronidazole alone, or in combination with azithromycin, induced clinical remission and reduced inflammatory markers; with the antibiotic combination being more effective.<sup>42</sup> In the same study, induction of remission was associated with suppression of microbiome diversity and shifts in species abundance.<sup>43</sup>

Diet is another major modifier of the gut microbiome and potential therapeutic in IBD. Exclusive enteral nutrition (EEN) is the first line induction treatment for active paediatric CD.<sup>44</sup> Cumulative evidence proposes that EEN ameliorates CD activity most likely through manipulation of the gut microbiome dynamics.<sup>45</sup> These included a decrease in bacterial diversity,<sup>24 45 46</sup> changes in species composition and a reduction of beneficial SCFA. Paradoxically, these effects coincided with reduction in colonic markers of inflammation and induction of clinical remission.<sup>24 46 47</sup>

Thus far, the limited efficacy of therapeutic strategies aiming to establish a healthy luminal microenvironment, and in contrast, the efficacy of drug and dietary therapies aiming to suppress the gut microbiome, support the hypothesis that suppression of selective inflammatory microbial species may be required to control gut inflammation in CD. Opposite

to that, therapeutic strategies aiming to create a normal or eubiotic gut microenvironment, including probiotics and FMT, hold better promise in UC.

### **Coeliac disease**

Although genetic predisposition occurs in 30-40% of the general population, only a small fraction of these individuals will develop coeliac disease (CoED) and many, years after first introduction of gluten. Findings from gut microbiome profiling studies, and basic and translational research point to the involvement of the gut microbiota in the underlying pathogenesis of CoED. However, the current evidence remains heterogeneous and findings often contradictory with the most consistent being a reduction of *Bifidobacterium* levels.<sup>48-50</sup> It remains also unclear from previous research whether a perturbed gut microbiota is implicated primarily in CoED pathogenesis, or if this is a secondary effect of altered gastrointestinal motility, excessive substrate availability from nutrient malabsorption, and in those with established disease, the effect of dietary modification during treatment with gluten free diet (GFD).

Using 16S rRNA amplicon sequencing and analysis of bacterial metabolites likely to be influenced by adherence to GFD, a recent study characterized the gut microbiome of children (4 to 16 years) with CoED.<sup>50</sup> The authors identified 11 distinctive bacterial taxa which composed a microbe signature distinctive to CoED with high diagnostic probability. Treatment with GFD also influenced microbiome composition with most of the bacteria and metabolites (e.g., butyrate) that differed between patients on GFD with newly-diagnosed CoED or healthy controls associated with nutrient and food group intake, and with biomarkers of gluten consumption.

Preclinical research offers opportunity to explore mechanisms by which the gut microbiota may be involved in disease pathogenesis. Inoculation of peripheral blood mononuclear cells with faeces from active and asymptomatic CoED patients increased TNF- $\alpha$  production and CD86 expression and decreased IL-10 cytokine production and CD4 expression compared with samples from healthy controls. Notably, specific *Bifidobacterium* strains suppressed this Th1 pro-inflammatory milieu, characteristic of CoED.<sup>51</sup> In gliadin-induced enteropathy murine models sensitized with interferon- $\gamma$ , *Bifidobacterium longum*

CECT 7347 attenuated the production of TNF- $\alpha$  and the CD4 mediated immune response and increased the tissue mRNA levels of NF $\kappa$ B and IL-10.<sup>52</sup> It has also been shown that bacteria could potentially reduce gluten immunogenicity by producing enzymes that effectively cleave proteolytic-resistant sequences in gluten peptides which activate Th1 response.<sup>53</sup>

There are very few preclinical and clinical trials which explored whether modulation of the gut microbiome can potentially improve outcomes in people with CoeD. Administration of *Bifidobacterium infantis* decreased Paneth cells and expression of  $\alpha$ -defensin-5 in duodenal biopsies of patients with active CoeD,<sup>54</sup> an effect which was associated with symptom improvement but which did not ameliorate abnormal intestinal permeability.<sup>55</sup>

Future research should explore the ability of microbiota signatures of unaffected siblings to predict risk of CoeD onset alongside other environmental factors. In previous research, healthy exclusively breastfed infants who were carriers of the HLA-DQ2 haplotype and had family history of CoeD, had less *Bifidobacterium*,<sup>56</sup> suggesting that genetic factors which impede the early colonization of the gut with species beneficial for human health, may potentiate the risk of development of CD.

Irrespective of their primary role in CoeD pathogenesis, disease-specific microbial signatures might be used as another adjutant, non-invasive biomarker to screen for CoeD. The observation that the abundance of fiber fermenting species and production of butyric acid diminish in children on GFD has possible implications for the dietary management of this population.

### **Irritable bowel syndrome**

Irritable bowel syndrome (IBS) is a functional gut disorder, characterised by chronic abdominal distension, pain and altered bowel habits. The increased risk of IBS development following an episode of infectious gastroenteritis and recent antibiotic use, and perturbation in intestinal immunity in mice following administration of colonic bacteria from IBS patients, points to the potential role of the gut microbiome in its underlying aetiology.<sup>57</sup>

Recent meta-analyses, including mainly studies in adults, demonstrated lower abundances of *Bifidobacterium* and *Faecalibacterium prausnitzii*,<sup>58 59</sup> in patients with IBS

compared to healthy controls. *Enterobacteriaceae* have been found to be enriched in patients with IBS,<sup>57 59</sup> while evidence regarding *Lactobacillus*, *Bacteroides* and bacterial diversity remains inconsistent within the available literature. A consistent finding, among paediatric studies, is the higher abundance of Gamma-proteobacteria in faeces of children with IBS and the absence of significant differences in diversity metrics compared with healthy controls.<sup>60</sup> <sup>61</sup> A recent cross-sectional study in children (7 to 12 years) further demonstrated the higher abundance of Gamma-proteobacteria and in addition showed that *Flavonifractor* and species from *Lachnospiraceae* were increased in faeces of children with IBS.<sup>62</sup> The higher abundance of the latter two taxa, along with bile acid metabolites and steroid metabolism pathways, were positively associated with the severity/frequency of abdominal pain. Higher levels of *Bacteroides*, *Haemophilus* and *Faecalibacterium* have also been linked to a less severe IBS phenotype.<sup>60</sup> Although the current literature highlight perturbations in the gut microbiome of people with IBS, the extent to which these are primary or secondary effects is still unclear.

A diet low in fermentable oligo-, di- and mono-saccharides and polyols (FODMAPs) is an established treatment for IBS.<sup>63 64</sup> Remarkably, the effect of a low FODMAP diet on the gut microbiome is counterintuitive with a decrease in levels of presumptive beneficial *Bifidobacterium*,<sup>65</sup> and other butyrate-producing bacteria.<sup>66</sup> The effect of a low FODMAP diet on the gut microbiome of children with IBS has not been studied extensively. Chumpitazi *et al.* demonstrated that symptomatic response to a 2-day low FODMAP diet was positively associated with higher levels of *Bacteroides*, *Ruminococcaceae* and *Faecalibacterium Prausnitzii* at baseline. Pathways of carbohydrate metabolism were also enriched in responders, thus suggesting that the efficacy of a low FODMAP diet might be positively influenced by host bacteria with increased fermentation capacity.<sup>67</sup>

The putative role of gut microbiome in IBS pathogenesis makes it an attractive target for therapeutic interventions. Non-absorbable antibiotics (i.e. rifaximin), have shown some effectiveness in adult patients with IBS-diarrhoea,<sup>68</sup> but in children the evidence is still unclear.<sup>69 70</sup> Probiotics may be effective in alleviating symptoms in patients with IBS, although in previous research efficacy varied between different bacterial strains and dosage and the absence of disease biomarker means that disease progression is based on self-reported gastrointestinal symptoms.<sup>71</sup> Irrespective of the limitations of current research only two

stains (*Lactocaseibacillus rhamnosus* GG and *Limosilactobacillus reuteri* DSM 17 938) have been proven to be effective in more than two RCT.<sup>72</sup>

Supplementation with soluble fibre reduced IBS symptom severity over placebo,<sup>73</sup> and so did psyllium after 6-week supplementation but without any alterations on the gut microbiome.<sup>74</sup> Interestingly, although administration of prebiotics was associated with increased *Bifidobacterium* levels, it did not result in alleviation of IBS symptoms, compared to placebo, further complicating the role of microbiome in IBS pathogenesis.<sup>75</sup>

## CONCLUSIONS

In contrast to the well-defined pathways by which pathogens cause infectious disease, the role of gut microbiome and early exposures to antibiotics in the onset, progression and management of non-communicable diseases is far more complex. The gut microbiome is altered in paediatric gut diseases (Figure 1), but it is currently too soon to draw any implications of current research findings in clinical practice. It is however possible that in the future gut microbiome may aid in disease differential diagnosis, prediction of disease outcomes or comprise a therapeutic target through drug, FMT and dietary manipulation (Figure 2).

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## **AUTHOR CONTRIBUTORSHIP**

KG is the guarantor, convened the author group and merged the individual sections to produce the final manuscript. All authors were involved in the preparation of this review article.

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## FIGURE LEGENDS

**Figure 1.** Consistent compositional and functional characteristics of the microbiome in paediatric gut diseases

**Figure 2:** Dietary, microbial and drug therapeutics aiming to modify gut microbiome composition or function in paediatric gut diseases

**Inflammatory bowel disease**

- ↑ *E. coli*, particularly adherent-invasive *E. coli*
- ↑ Proteobacteria

**Necrotizing enterocolitis**

Before disease onset: ↑ Proteobacteria

**Intestinal failure**

- ↑ Proteobacteria, especially *Enterobacteriaceae*
- ↑ *Lactobacillus*, presence of *Lactobacillus mucosae*

**Irritable bowel syndrome**

- ↑ *Enterobacteriaceae*
- ↑ Gamma-proteobacteria
- ↑ *Flavonifractor*
- ↑ *Lachnospiraceae*



**Inflammatory bowel disease**

- ↓ Bacterial diversity & stability
- ↓ Firmicutes, *F. prausnitzii*, *Bifidobacterium*, *C. coccoides*, *C. leptum*

**Coeliac disease**

- ↓ *Bifidobacterium*
- On gluten free diet: ↓ butyric acid

**Necrotizing enterocolitis**

- ↓ Bacterial stability
- ↓ *Bifidobacterium*

**Intestinal failure**

- ↓ Bacterial diversity
- ↓ Short chain fatty acids
- ↓ Firmicutes
- ↓ Bacteroidetes

**Irritable bowel syndrome**

- ↓ *Bifidobacterium*
- ↓ *F. prausnitzii*



