

# Gut microbiota in celiac disease

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## Abstract

Celiac disease (CD) is an autoimmune gastrointestinal disease triggered by dietary gluten, occurring in genetically predisposed individuals. Currently, a gluten-free diet is the only current evidenced-based treatment for CD. With the growing prevalence of this condition worldwide, adjuvant therapies are needed. We understand that there are several factors that influence the pathogenesis of the condition. There is a complex interplay between genetics, environmental triggers, the immune system and gut microbiota. Recently, there has been a growing focus on the significance of gut microbiota in several autoimmune-based conditions. In particular, there has been much research involving the role of microbial flora and CD. Here, in this mini-review, we highlight the importance of gut microbiota and the symbiotic relationship with the host, introduce key factors that influence the development of the intestinal flora in early colonization, and ultimately explore its role in the pathogenesis of CD.

**Keywords** Gastrointestinal microbiota, celiac disease, gluten

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## Background

Celiac disease (CD) is an autoimmune disease of the small intestine, triggered by the ingestion of dietary gluten in genetically susceptible individuals [1]. The reported prevalence is between 0.5-1% in Europe and North America, with higher rates recorded in Middle Eastern and Asian-Pacific countries [2]. There is a higher incidence of CD in patients with first-degree relatives and patients with other autoimmune disorders, including type 1 diabetes and autoimmune thyroid disease [3].

A strong genetic predisposition is associated with CD: both human leukocyte antigens HLA-DQ2 and HLA-DQ8 are highly prevalent in patients with the condition [4]. There are several phenotypical presentations of CD as per the 2013 Oslo classification: classical, non-classical, subclinical, potential, and

refractory [5]. Whilst serological testing, including anti-tissue transglutaminase 2 and anti-endomysium antibodies, is an excellent diagnostic tool for CD, the gold-standard diagnosis is through a small bowel biopsy [6]. Classic histological criteria involve the degree of intraepithelial lymphocytes, crypt hyperplasia, and villous atrophy. The mainstay of treatment involves lifelong avoidance of gluten products [7].

The pathophysiology of CD involves a complicated relationship between genetic predisposition, the innate and adaptive immune system, environmental triggers and the gut microbiota [8]. Numerous studies have highlighted an association between gut microbial changes and CD, with further studies exploring the impact of gut microbiota in both upregulating and downregulating underlying CD immunology.

The main aim of this mini-review is to discuss our current knowledge of the relationships between the gut microbiota and CD, how environmental factors influence this, and the role of future targeted therapies in CD, including probiotics. PubMed was used to search for all studies published from Jan 2005 to November 2023, using key words such as "Celiac Disease" and "Gut Microbiota", "Celiac Disease" and "Gastrointestinal Microbiome". The search was limited to articles published in English, and included case studies, case-control and cohort studies, literature reviews, systematic reviews and meta-analyses.

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## Gut microbiota

Growing evidence suggests a possible interaction involving the gut microbiota in both the pathogenesis and clinical symptoms of CD. The human microbiome consists of a

wonderfully multitudinous, diverse and complex population of micro-organisms, including bacteria, viruses, fungi and protozoans. Unique to each individual, the early composition of gut microbiota is heavily influenced by both genetic and environmental factors, such as the type of delivery, nutrition and diet in infancy, and the use of antibiotic therapy [9].

*In utero*, placental hormones, including progesterone and estrogen, influence the regulatory mechanisms of the brain–gut axis and the immune activation of intestinal mucosa. Typically, microbiome compositions occur between the first and third trimesters, with associated increases in *Akkermansia*, *Bifidobacterium*, and *Firmicutes* [10].

By the age of 3 years, the gut microbiota will stabilize, but will still be sensitive to lifestyle and host factors. The role of the gut microbiome in influencing human physiology is largely symbiotic, including the development and regulation of the immune system, nutrient and drug metabolism, protection against pathogens, and maintenance of the gut mucosal barrier. A healthy intestinal flora consists of *Firmicutes*, *Bacteroides*, *Proteobacteria*, and *Actinomyces* [11].

Changes in gut microbial composition, or dysbiosis, have been associated with numerous chronic inflammatory-driven diseases, including inflammatory bowel disease, liver disease, and a range of neurodegenerative conditions such as Parkinson's disease [11]. Recent research has explored and supported the ongoing notion that commensal changes may influence the pathogenesis of CD.

## Genetics

Genetics may play a highly important role in the development of CD. With a high negative predictive value, over 90% of patients with CD are positive for the HLA-DQ2 genotype, with HLA-DQ8 covering the remaining 10%. In particular, numerous studies have evaluated the relationship between HLA-DQ genotype and composite changes in gut microbiota. Infants at family risk of CD showed variances in early-stage gut microbial composition. A prospective study looking at infants who had high genetic risk (HLA-DQ2 carriers) or low genetic risk (non-HLA-DQ2/8 carriers) for CD occurrence, found that the high-risk group had a different early gut microbial composition: infants with the HLA-DQ2 genotypes had significantly higher proportions of *Firmicutes* and *Proteobacteria*, and lower proportions of *Actinobacteria*. Quantitative real-time polymerase chain reaction also revealed lower numbers of *Bifidobacterium* species in infants in the high-genetic-risk group [12].

Olivares *et al*, in a different study, noted that the microbiota of healthy infants over time showed an increase in bacterial diversity, compared with high-risk CD infants [13]. Furthermore, infants who developed CD showed significantly lower levels of secretory IgA. IgA, the first line of defense within the intestine, is synthesized by gut-associated lymphoid tissue. It has an important role in the control of commensal bacteria colonization [14]. It is possible that changes in the early gut microbial flora influence immunological homeostasis, and

subsequently strengthen the risk of increasing the autoimmune profile. Although larger population studies are required, the findings suggest that alterations in the early trajectory of gut microbiota in infants at CD risk could influence immune maturation and subsequent predisposition.

Other studies have also shown a link between HLA genotype and microbial composition in high-risk infants, in particular a higher prevalence of *Bacteroides* species [15,16]. Finally, a multi-omics analysis revealed a lower abundance of *Coprococcus* species in high-genetic-risk infants at 4–6 months of age. Reduced *Coprococcus* levels have also been described in the gut flora of patients with an enhanced risk of developing CD [17].

With new techniques and the introduction of genome-wide association studies, further discoveries of non-HLA genotypes and susceptibility to CD development have been identified. Although carrying HLA-DQ2 and HLA-DQ8 is almost a requirement for developing CD, more than 30% of the population are carriers, but not all are affected [18]. It is therefore plausible to suggest that other genetics may be involved. Since 2007, more than 40 loci outside the HLA region have been associated [19]. Sharma *et al* noted that key genotypes involved in CD pathogenesis included TAGAP, IL18R1, RGS21, PLEK, and CCR9. They also noted that 2 further regions in CTLA4 and LPP were associated with the development of anti-tissue transglutaminase [20].

## Immune system and intestinal permeability

Regulation of the immune system by gut flora is well established, and plays a critical role in the function of both the innate and adaptive immune system [21]. As highlighted by Rossi *et al*, changes in gut microbiome may contribute to various chronic immune disorders, such as CD. Several hypotheses have been postulated by the research community as to how the microbiota may directly influence the immune system in CD [22]. Gut microbiota may upregulate proinflammatory or downregulate anti-inflammatory mediators and metabolites. Studies have shown a relationship between microbes and the immune state. *In vitro* studies of *Bifidobacterium longum* (*B. longum*) and *Bifidobacterium bifidum* were investigated regarding cytokine production [23]. Both strains, in combination with feces from both active CD and symptom-free CD, were also shown to restore the proinflammatory cytokine profile through lower production of tumor necrosis factor (TNF)- $\alpha$  and interferon (IFN)- $\gamma$ . These strains are thought to possess anti-inflammatory properties through the induction of interleukin (IL)-10. This in turn inhibits the production of IFN- $\gamma$  and TNF- $\alpha$ , therefore regulating the balance between CD4 and CD8 T-helper cells [24,25].

A key role of the gut flora is intestinal barrier modulation. Zonulin, synthesized by liver and intestinal cells, is a protein that modulates the permeability of junctions between cells [26]. It essentially regulates the “leakiness of the gut”. Gliadin has been shown to bind to chemokine receptor CKCR3 on epithelial cells, increasing intestinal permeability through

the excessive release of zonulin [27]. This is significant, as it further enables passage of gliadin to the gut mucosa, triggering the innate immune response and worsening the inflammatory state.

In animal experimental models, *Bacteroides fragilis* (*B. fragilis*) has been implicated in the regulation and breakdown of the intestinal epithelial layer, with the pathogenicity related to the expression of virulence factors and other proteolytic enzymes [28,29]. Sanchez *et al* investigated the role of *B. fragilis* strains in Caco-2 cells [30]. They noted clones were more frequently identified in CD patients than in control subjects, with no change in differences following a gluten-free diet. Increased levels of *B. fragilis* strains expressing virulence genes coding for metalloproteases were also recorded. These strains showed high gelatinase activity and gliadin-hydrolyzing activity, and generated immunogenic peptides that upregulated inflammatory cytokine production, e.g., TNF- $\alpha$ . The results suggest increased permeability of the gut, and significantly higher levels of *B. fragilis* with metalloprotease activities may well be a factor in the development of CD. However, further *in vitro* and *in vivo* studies are needed to confirm this hypothesis.

Short-chain fatty acids (SCFAs), e.g., butyric acid and acetic acid, are important in regulating intestinal permeability. SCFAs are the main metabolites produced by the anaerobic fermentation of dietary fibers by gut commensals. Their role is crucial in maintaining a tight intestinal barrier integrity junction and T-cell differentiation [31]. However, the high fatty acid concentration is harmful, because of the increased oxidative stress and upregulation of the proinflammatory state. Its imbalance is linked to several diseases, including Crohn's disease and multiple malignancies [32,33]. Baldi *et al* assessed the serum free fatty acid profile in patients with CD. They found a higher percentage of butyric acid and a strong positive association in CD patients compared with healthy controls [34]. Butyric acid is important; it is an energy source for the intestinal epithelium and regulates intestinal homeostasis. However, in CD, because of intestinal epithelial dysfunction, it is not consumed and subsequently higher volumes enter the bloodstream. This was therefore suggested as a potential early biomarker for patients with CD.

## Environmental factors

Alongside genetic susceptibility in predisposed individuals, environmental factors are also a vital component in the pathogenesis of CD—in particular, shaping the nature and diversity of gut microbiota in the early years of life. Here, we describe several modes by which the composition of the intestinal microbiome colonization is affected.

### Type of feed

Breastfeeding is known to aid in the healthy colonization of the gut microbiota in many different conditions [35]. The benefits of human milk are multifold, including commensal

growth, e.g., *Bifidobacteria*, prevention of pathogenic agents, e.g., *Clostridioides difficile* (*C. difficile*), transmission of immunoglobulins and ensuring adequate nutrients for growth [36]. However, the role of breastfeeding in the development of CD is controversial. A 2006 systemic review of 6 case-control studies noted breastfeeding as protective and reducing the risk of developing CD [37]; however, a 2016 systematic review found that neither the duration of breastfeeding nor breastfeeding at the time of gluten introduction was effective in preventing later development of CD. Furthermore, a delayed introduction of gluten during weaning was again not effective [38]. A 2019 systematic review of the relationship between the consumption of human milk, duration and intensity concluded that there is only limited evidence to support an association with CD [39].

Fascinatingly, exposure to exclusively formula milk in infants with 1st-degree CD relatives is associated with a lower abundance of *Staphylococcus epidermis* and enhanced levels of *Ruminococcus gnavus* and *Lactospiraceae* bacterium. These 2 strains, respectively, have been associated with allergic disease and colonic inflammation [40].

### Mode of delivery

The mode of infant delivery is considered a fundamental determinant of early gut microbiota [41]. Vaginal deliveries are widely hypothesized to aid the development of a wider diversity of gut flora, ensuring the vertical transmission of pro-beneficial bacteria such as *Bacteroides* and *Bifidobacteria* [42]. Cesarean section (C-section) delivery is associated with increased host susceptibility to chronic inflammatory diseases, including rheumatoid arthritis and inflammatory bowel disease [43]. C-section-born infants were noted to have a smaller diversity of gut flora [44]. Observational studies have noted that dysbiosis arising from C-section delivery may lead to a higher risk of CD in young patients [43,45]. Leonard *et al* noted C-section delivery is associated with lower levels of several species of *Bacteroides* and *Parabacteroides* at all tested time points, with *Enterococcus faecalis* levels raised at 3 months after birth. Beneficial species, including *Bacteroides vulgatus* and *Bacteroides dorei*, were also lower [17]. Healthy levels of these bacteria have been reported to have advantageous effects on immune function [46]. However, a 2022 meta-analysis of 11 observational studies identified no association between C-sections and CD in offspring [47]. Whilst variances in gut microbiota as a result of delivery mode may be present, it is still controversial whether there is an association between CD risk and birth mode.

### Antibiotics

Antibiotic therapy and exposure can impact the composition of the gut flora and influence the development of chronic diseases [48]. Patients who have an early intestinal infection treated with antibiotics can develop intestinal dysbiosis. The

changes in gut microbiota may predispose to a higher risk of CD. In Norwegian and Danish case-control studies, both found that antibiotic exposure in the first year of life was positively correlated with a diagnosis of CD in later years [49]. There also seems to be a dose-dependent relationship between the number of dispensed antibiotics and CD risk [50]. Additional population case-control studies in Swedish, Italian and North American children revealed a strong association between antibiotic therapy and the development of CD [49,51,52]. However, none of these studies were able to delineate whether antibiotic use was simply a response to the manifestation of undiagnosed disease. A large multinational prospective cohort study (TEDDY) noted that the most prescribed antibiotics during the first 4 years of life, regardless of geographic region, were not associated with the development of autoimmunity for CD children at increased genetic risk. These antibiotics included cephalosporins, penicillins, and macrolides [53]. Furthermore, no association has been established between antibiotic consumption during pregnancy and the risk of CD, and the evidence is conflicting [54]. Nevertheless, it is still best clinical practice to prescribe antibiotics for patients with bacterial infections. Whilst there may be variances in gut microbiota as a result of delivery mode, it is still controversial whether there is an association between CD risk and birth mode.

## Infections

Large-scale population cohort studies have identified early infections as potential causes of the pathogenesis of CD [55]. Several enteric viral strains have been identified as possible triggers for the development of CD, including rotavirus, adenovirus and reovirus [56]. Viral infections early in life could affect the maturation of the mucosal immune system, reduce the breakdown of tolerance against gluten in high-risk patients, and cause long-term differences in the gut microbiota [57]. Repeated gastrointestinal infections during the first 6-18 months of life may also be associated with an increased risk of CD [58]. Frequent rotavirus infections may augment CD autoimmunity in childhood in genetically susceptible patients [55,59]. In summary, certain infections may be involved in developing CD; however, since there are no studies investigating the impact of gastrointestinal infections on gut microbiota, this could be a potentially important research avenue with a view to developing targeted treatments.

## Gluten

The only known treatment for CD is a gluten-free diet. This can be expensive and must be strictly adhered to prevent relapse of symptoms. The immune response in CD to gluten is complex. Gluten is a key protein found in wheat, barley and rye. A central component of gluten is gliadin, an insoluble protein indigestible to gastrointestinal brush border enzymes. When ingested, gliadin fractions react with tissue transglutaminase

(tTG) within the gastrointestinal lumen, leading to tTG-induced deamidation. This process results in negatively charged glutamic acid molecules, which are subsequently presented by major histocompatibility complex class II molecules DQ2 and DQ8. Recognized as pathogenic by CD4+ T-cells, a humoral immune response occurs, with the production of antibodies against gliadin, tTG and stimulation of proinflammatory cytokines such as TNF- $\alpha$ , IFN- $\gamma$ , and IL-21 [1]. IFN- $\gamma$  and IL-21 induce IL-15, leading to the proliferation of interepithelial lymphocytes. Tissue remodeling and chronic inflammation of the small intestine leads to the classic histological appearances mentioned above, including crypt hyperplasia, villous atrophy, and lymphocyte infiltration [7]. This in turn leads to the classical symptoms of CD, e.g., diarrhea, abdominal bloating and abdominal pain.

Limited studies have classified strains that could be involved in gluten metabolism. Caminero *et al* cultured several strains, with 94/144 strains able to metabolize gluten and 61 strains showing proteolytic activity against gluten protein. The strains classified were from the *Firmicutes* and *Actinobacteria* phyla, e.g., *Lactobacillus*, *Streptococcus*, *Staphylococcus*, *Clostridium*, and *Bifidobacterium* [60]. Significantly, *Lactobacilli* may detoxify gliadin peptides and dampen other immunogenic peptides [61]. Moreover, *Bifidobacterium lactis* may neutralize gliadin toxicity, with a reduction in epithelial permeability in Caco-2 cells [62]. These results suggest that these strains may have potential in therapies to aid gluten metabolism, e.g., in the form of probiotics.

Gluten-free diets may also affect the impact of gut microbiota. Research has noted that gluten-free diets consumed by both CD patients and controls can significantly affect the populations of *Lactobacillus* and *Bifidobacterium* species [63,64], whilst harmful levels of *Enterobacteriaceae* and *Escherichia coli* have also been seen. *Veillonellaceae*, a gram-negative bacterium belonging to the *Firmicutes* phylum, is known for carbohydrate and starch metabolism. Bonder *et al* noted that a 4-week gluten-free diet resulted in lower levels of *Veillonellaceae* [65]. These results noted for the bacterial strains may be influenced by a reduced carbohydrate intake, i.e. polysaccharides associated with a gluten-free diet. A main energy source for gut microbiota, these polysaccharide compounds reach the distal colon undigested. The genome of these bacteria encodes enzymes that thrive on non-digestible carbohydrates, resulting in favorable growth and competitive advantage for colonization. When this harmony is disrupted, it may lead to the expansion of pathogenic bacteria and alterations in mucosal immunity.

However, any extrapolation of data from one population to the other is challenging. Gut microbial composition and metabolic activity are unique to each individual, and there are many factors associated with their variances. Limitations related to sample size, technical analysis and variations of CD presentation in patients make extrapolation difficult [66]. Analysis at a cell level may be helpful for researchers to understand and determine physiological changes that may be influenced in future adjuvant treatments.

## Future treatments

### Probiotics

Presently, the only treatment for CD is a strict lifelong gluten-free diet. Whilst a gluten-free diet can lead to remission of symptoms, gut dysbiosis may still be predominant [67]. Therefore, other modes of treatment are being investigated for CD. The World Health Organization defines a probiotic as live microorganisms that are administered in adequate amounts to confer a health benefit on the host [68]. The advantages of probiotics may include recolonizing the composition of gut flora, modulating the immune response and boosting pathogen resistance [69].

Probiotics with a gluten-free diet are highly effective in reducing clinical diarrhea in CD patients [69]. The most commonly studied probiotics involve *Lactobacilli* and *Bifidobacterium* species. As stated previously in this review, immunogenic gluten peptides are harmful and contribute to the pathophysiology of CD. Several studies have reported the ability of probiotics to dampen the immunogenicity of these peptides [70-72]. Lindfors *et al* noted that probiotics, including strains of *Lactobacillus fermentum* and *Bifidobacterium lactis*, play a protective role against the toxic effects of gliadin in human colon Caco-2 cells [73].

Similarly, further studies of probiotic mixes including *Lactobacilli* and *Bifidobacteria* strains showed positive results. The results suggested that this combination reduced the toxicity of gliadin after digestion and inhibited its inflammatory effects on intestinal epithelial cells [74]. Finally, a double-blinded randomized control trial involving *Bifidobacterium* species, including *B. longum*, in combination with a gluten-free diet resulted in reduced secretory IgA levels [75].

However, most studies have involved animal models, with limited human trials. In mouse models expressing human DQ8, strains including *Lactobacillus casei*, *Lactobacillus paracasei* and *Lactobacillus fermentum* reduced TNF- $\alpha$  secretion and villous blunting [76,77]. The data from both animal and limited human studies are promising; however, further large-scale trials are needed to evaluate the safety and efficacy of treatments. These trials must include a standardized protocol, dose regimen, length of treatment and homogeneity of patient demographics [78].

### Prebiotics

Prebiotics are defined as a substrate that is selectively utilized by host microorganisms, conferring a health benefit. They are naturally present in foods, including garlic and onions. Prebiotics have been studied for certain health benefits, including cardiovascular health and immune modulation. The most commonly studied prebiotics are the soluble fibers inulin, fructooligosaccharides, galactooligosaccharides, and human milk oligosaccharides [79]. However, only a few pilot studies have investigated the effectiveness of prebiotics in intestinal inflammation [80,81].

In the limited studies assessing prebiotics in CD, the results are promising. In a double-blind randomized control

trial, oligofructose-enriched inulin (Synergy 1) was given to pediatric CD patients following a gluten-free diet. Both gut microbiota and SCFAs were evaluated. After the intervention period, *Bifidobacterium* counts increased significantly, and an increase in acetic acid and butyrate levels was noted in patients taking prebiotics [82]. Iron deficiency anemia is a common manifestation of CD. A similar trial concept evaluated the effect of Synergy 1 on iron homeostasis in non-anemic children with CD, in association with a gluten-free diet. Significantly, serum hepcidin concentration, a key regulator for duodenal iron, decreased after the intervention, whereas no significant difference was observed in the placebo group. This may signify increased absorption of iron within the small bowel, which is a substantial issue in CD. However, since this trial had a small cohort size and short intervention duration, the results will need to be validated in a larger trial, and adult CD patients [83].

Both prebiotics and postbiotics may be highly effective alongside a gluten-free diet in CD patients, but cautious interpretation is required. The use of neither treatment has yet been justified in clinical practice, and further research and recognition of side-effects is needed.

### Fecal microbiota transplantation (FMT)

FMT involves the infusion of feces from a healthy donor to treat disease arising from gut dysbiosis [84]. It is an effective treatment for *C. difficile* infection [85] and is being investigated in a range of other inflammatory conditions including Crohn's disease and ulcerative colitis. Only a few case reports have reported the role of FMT in CD. One case study of a 68-year-old male patient with CD and superimposed *C. difficile* infection noted the resolution of symptoms following FMT. Remarkably, duodenal biopsies taken 6 months post-infection noted complete villous atrophy recovery [86].

### Concluding remarks

There seems to be a complex relationship between CD and gut microbiota. A host of different factors seem to influence the early colonization and development of gut flora within the context of CD. Multiple studies have noted variances in gut microbial abundancies in response to several stimuli including genetics, environmental triggers and dietary gluten. Research regarding adjuvant therapy with a gluten-free diet is further required, but initial results are promising. We suggest further high-quality clinical studies, particularly in regard to probiotic therapy, in the treatment of this chronic condition.

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