REVIEW

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Celiac Disease and Gut Microbiota: What Do We Know so Far?

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ABSTRACT

Gut microbiota (GM) is a complex microenvironment characterised by intricate interactions, which might play a pivotal role in the pathogenesis of many autoimmune diseases, including celiac disease (CD). DDysbiosis of gut bacteria can have a wide range of effects; however, whether it is a cause or a consequence of certain diseases and how it evolves during disease progression remains an area of active research. Celiac disease patients appear to have characteristic microbiota patterns, including low levels of beneficial species and high levels of potentially pathogenic species. The use of pro-, pre-, syn- and post-biotics to modulate the GM requires more systematic investigation, to determine the impact of specific species, the optimal dosage and the treatment duration needed to achieve desired results without adverse reactions. Additionally, osteoporosis in the course of CeD ease warrants further investigation. Many factors may contribute to its pathogenesis, including GM. This review summarizes recent literature concerning the role of GM in CeD, highlighting both consistent findings and areas of ongoing debate.

Key words: celiac disease – gut microbiota – probiotics – prebiotics – osteoporosis.

Abbreviations: APC: antigen-presenting cell; ArR: aryl hydrocarbon receptor; CeD: celiac disease; GFD: gluten free diet; GM: gut microbiota; Ig: immunoglobulin; IFN: interferon; IL: interleukin; PPI: proton pumb inhibitor; SIBO: small intestine bacterial overgrowth; SCFA: short-chain fatty acid; TG2: tissue-transglutaminase 2; TNF: tumor necrosis factor; Trp: tryptophan.

INTRODUCTION

Autoimmune diseases have a rising prevalence and their complex aetiology is not always exactly understood. The implicated factors are genetic and environmental [1]. The Gut microbiota (GM) has emerged as a potential contributing factor and an increasing number of studies focus on it [1,2].

Celiac disease (CeD) is an autoimmune enteropathy, affecting the small intestine, with a prevalence of 1-1.5% of the worldwide population [3-6]. In the 1940s, Dicke first described CeD and in 1954, Paulley described its histologic findings. In 1969, the European Society for Paediatric Gastroenterology and Nutrition (ESPGHAN) published the first guidelines for the diagnosis of CeD, with the last update in 2020 [7, 8]. Celiac disease is triggered by gluten consumption in genetically predisposed individuals (HLA DQ2, DQ8) [9, 10]. It has a broad clinical spectrum, including intestinal (e.g., diarrhoea, constipation, abdominal pain/distension) and extra-intestinal symptoms (e.g., anaemia, malabsorption, dermatitis herpetiformis, osteopenia), or can be asymptomatic [8, 11, 12]. Environmental factors, including birth method, early-life gastrointestinal infections, antibiotic or proton pump inhibitor (PPI) use, maternal iron supplementation, and breastfeeding practices, have been studied for their potential association with CeD, although findings remain conflicting in several areas [3, 10, 13, 14].

Dysbiosis is the loss of balance between pathogenic and protective microbes of the intestinal tract [9]. Research has been focused on the association between microbiota changes, dysbiosis, and the pathogenesis and treatment of CeD [4, 14]. Celiac disease itself, along with gluten-free diet (GFD), may influence gut microbiota composition [4]. However, despite the implications of GM's role in CeD, little data exist about the in vivo use of probiotics for CeD management [15-17].

This review aims to summarise the available literature on the complex associations between CeD and GM, including current knowledge on the role of pro-, pre- and synbiotics for CeD and the potential involvement of the GM in the coexistence of CeD and osteoporosis.

A comprehensive literature search was conducted in the MEDLINE/PUBMED database up to March 2024 using the search terms: "celiac disease," "gut microbiota," "intestinal microbiota," "osteoporosis," "probiotics," "prebiotics," and "synbiotics". English-language publications from the last ten years were included, focusing on human studies or translational findings relevant to humans and research focused on CeD and its interaction with the GM. Two independent reviewers performed the selection process by screening titles and abstracts, followed by full-texts review for eligibility. A formal quality assessment was not conducted, consistent with the exploratory nature of a narrative review, but narrative judgments on study quality were applied during data extraction. In total, 40 studies were included (24 reviews and 16 original articles), summarised in Tables I and II.

Table I. Summary of the included studies.

Study (Author-year) Study type	Model	Intervention	Population	Key findings	Implications
Xu et al. [1] 2022 Mendelian Randomization	Human	-	>24,000	The role of Bifidobacterium in CeD appears to be conflicting, as some studies suggest it may be either harmful or protective.	Host genetic background seems to influence GM composition, which may subsequently impact the risk of developing CeD.
Gonzalez-Garcia et al. [21] 2023 Mendelian Randomization	Human	-	18,340; Immunochip data	Higher abundance of Proteobacteria was associated with increased CeD risk, while Ruminococcaceae and Lachnospiraceae might play role as cause or consequence.	These findings suggest a potential causal and bidirectional relationship between gut microbiota and CeD, based on MR data.
Li et al. [30] 2023 Mendelian Randomization	Human	-	18,340 in MR; 7,824 GWAS; 4,533 CeD cases	A causal association was identified between higher Bifidobacterium levels and increased risk of CeD, whereas Subdoligranulum showed a protective effect.	This supports the hypothesis of a two-way association between gut microbiota composition and CeD development.
Francavilla et al. [33] 2018 Clinical trial	Human	Probiotic	109 CeD 55 Placebo	The probiotic study is effective in improving the severity of IBS-type symptoms in patients with CeD adherent to a strict GFD.	Microbiota-mediated symptom relief Secondary effect the steady and persistent increase of the bifidobacterial
Drabinska et al.[36] 2018 Clinical trial	Human	Prebiotic	34 CeD children	Prebiotic intake led to significantly increased vitamin D levels in children with coeliac disease. (optimal status increased from 17% to 46%)	The improved vitamin D status may be linked to GM modulation, supporting the use of prebiotics for micronutrient absorption.
Drabinska et al.[35] 2018 Clinical trial	Human	Prebiotic	34 CeD children 16 Placebo	Supplementation with Synergy 1 (prebiotic) increased Bifidobacterium levels and SCFAs in children with CeD and decreased Lactobacillus	The increase in SCFAs is associated with improved gut health, supporting the use of prebiotics as a supportive therapy in CeD.
Ali et al. [16] 2022 Clinical trial	Human	Probiotic + GFD	170 CeD children	Combined probiotic (Clostridium butyricum and Bifidobacterium) and GFD significantly decreased GI symptoms compared to GFD alone. Decreased diarrhea	C. butyricum and Bifidobacterium may be clinically effective in alleviating CeD-related gastrointestinal symptoms.
De Palma et al. [28] 2012 Observational	Human	-	232 infants	Breastfeeding was associated with increased Bifidobacterium, C. leptum group, B. longum, and B. breve., while formula feeding and HLA-DQ2 genotype were associated with increased Bacteroides fragilis, C. coccoides-E. rectale group, E. coli, and B. lactis.	Early feeding patterns and genetic predisposition shape the infant gut microbiota, which may influence CeD risk later in life.

Table I (continued)

Palmieri et al [32] 2022 Observational	Human	-	46 CeD 30 Controls	CeD patients had lower levels of Bifidobacterium and higher levels of Bacteroides compared to controls.	A gluten-free diet only partially restores the gut microbiota composition in CeD patients.
Lamas et al. [22] 2020 Expretimental	Animal & Human	Dietry Tryptophan	Mice models (in vivo) & CeD biopsies (ex vivo)	A Trp-enriched diet increased Lactobacillus, Ruminococcus.	Diet may modulate GM and immune response through AhR pathway activation.

CeD: celiac disease; GWAS: genome-wide association study; GM: gut microbiota; IBS: irritable bowel syndrome; Trp: tryptophan; AhR: aryl hydrocarbon receptor; MR: mendelian randomization; GFD: gluten free diet; SCFA: short chain fatty acids.

Table II. Summary of the reviews included

Category	Subtheme	Key findings	Reference
Microbiota-Modulating therapies	Probiotics	- *Lactobacillus*, *Bifidobacterium*, *S. boulardii*, and VSL#3 improve GI symptoms, restore gut barrier integrity, and reduce inflammation. - Strain-specific effects observed in both pediatric and adult CeD. -*B. breve* reduced TNF-α levels in pediatric CeD after 3 months, although not sustained post-intervention. *B. longum* decreased cytokine production and CD4+ T cells in animal models. - *L. casei* and *L. rhamnosus GG* demonstrated mucosal protection in rats. Meta-analysis showed benefits primarily when GI symptoms were measured using GSRS.	[3, 9, 11, 12, 13, 19, 23, 24, 17]
	Prebiotics & FMT	- Inulin and other prebiotics increased short-chain fatty acids (e.g., propionate, butyrate), especially *Bifidobacterium* and *C. leptum* in children. - One trial showed a 42% increase in plasma vitamin D after 3 months of Synergy 1. - FMT showed symptom improvement in a case of refractory CeD with *C. difficile* infection.	[14, 19, 31]
Microbiota composition & CeD	Effect of GFD	- GFD alters gut microbiota due to reduced polysaccharide intake, leading to decreased *Bifidobacteria*, *Lactobacillus*, and increased *Proteobacteria*. -Even after 2 years of GFD, beneficial bacteria remain low in some cases. -In healthy individuals, GFD may reduce microbial diversity and increase *Enterobacteriaceae*.	[4, 11, 15, 26]
	CeD & Associated dysbiosis	- Increased *Proteobacteria*, decreased *Firmicutes*, *Bacteroidetes*, and *Bifidobacterium* are hallmarks of CeD-associated dysbiosis. - CeD patients with GI symptoms show overgrowth of *Pseudomonadota*, while those with DH have higher *Bacillota*. --- - Reduced microbial diversity and lower IgA coating were noted.	[4, 7, 9, 20, 26]
Pathogenesis & Risk factors	Genetics & Immune- Microbial interactions	- HLA genotypes influence microbiota profiles and CeD risk. - Microbial peptides can mimic gliadin epitopes, activating immune responses. - Reovirus infections may contribute to abnormal immune responses to dietary antigens. - Oral bacteria such as *Rothia*, *Actinomyces*, and *Streptococcus* degrade gliadin in CeD patients.	[14, 20, 25]
	Early-life exposures	- Cesarean delivery, formula feeding, and antibiotics (especially amoxicillin) in infancy correlate with increased CeD risk due to microbiota disruption. -Breastfeeding promotes *B. longum*, *B. breve*, and *Cl. leptum*; formula-fed infants have more *Bacteroides*. - Genetic risk modifies microbial colonization patterns. -Timing and dosage of antibiotic exposure are crucial.	[7, 10, 14, 18, 19, 23, 24, 25, 27]
Nutritional & Systemic effects	Bone health & micronutrients	- Gut microbiota influences absorption of calcium, magnesium, and phosphorus. - Low BMD (30–60%) and osteoporosis (18–35%) are common in newly diagnosed CeD. -Vitamin D affects the gut-immune axis and its absorption may be modulated by microbiota composition. - No direct evidence yet linking probiotic use to improved BMD.	[5, 38]

GI: gastrointestinal; FMT: fecal microbiota transplantation; BMD: bone mineral density; DH: dermatitis herpeteformis; GSRS: gastrointestinal symptoms rating scale. For the rest of abbreviations see Table I.

PATHOPHYSIOLOGY

Celiac disease is an immune-based disease with a complex interaction between genetics, environmental factors and GM. Gluten consumption in genetically predisposed individuals is central to its pathogenesis [3, 4, 14]. Gluten, which consists of proteins, is found in wheat, barley and rye [3]. Gliadin (soluble), a component of gluten, together with glutenin (insoluble), breaks down into peptides, which cause the production of zonulin [14]. Zonulin increases intestinal permeability, inducing gluten entry

into the lamina propria [18, 19]. Gluten peptides can translocate into the lamina propria, largely via transepithelial transport mediated by their binding to secretory immunoglobulin A (IgA) [20]. When gliadin peptides reach the lamina propria, they are deamidated by tissue-transglutaminase 2 (TG2). TG2 is an enzyme expressed in many cell types, released into the extracellular space during inflammation and into tissues under mechanical stress [20]. Deamidation of gliadin facilitates its binding with HLA-DQ2/DQ8. These HLA haplotypes are expressed on the antigen-presenting cells (APCs); deamidated gliadin peptides are then presented by APCs (macrophages, dendritic and B cells) to CD4 Th1 cells. This causes, through inflammatory cytokines [interferon (IFN)-γ, interleukin (IL)-12 and tumor necrosis factor (TNF)-α], a rise in lymphocytes, increased expression of NK receptors and apoptosis of enterocytes. The end result is inflammation of the intestinal mucosa, resulting in crypt hyperplasia and villous atrophy [4, 11, 13, 14, 19, 20]. B-cells also contribute to the pathogenesis of CeD. They serve as key APCs and likely reinforce the immune response internalising TG2-gluten complexes, releasing deamidated gluten peptides (which bind to HLA-DQ2/-DQ8) and activating gluten-specific T-cells [20]. B-cell clones produce IgA autoantibodies against TG2, highly sensitive and specific markers of active CeD [20]. The key role of TG2 in the pathogenesis of CeD was highlighted in a phase 2 clinical study, where the oral TG2 inhibitor ZED 1227 dose-dependently protected patients with CeD from gluten-induced mucosal damage and inflammation [20]. The circulation of gliadin peptides in the blood amplifies the inflammatory response and contributes to extra-intestinal symptoms [4, 14].

The high prevalence of CeD among family members and identical twins shows that genetic predisposition is a major factor in disease occurrence. HLA-DQ2 is present in more than 90% of patients with CeD. HLA-DQ2.5 is encoded by the DQA allele DQA1*0501 and the DQB allele DQB1*0201, while the rarer HLA-DQ2.2 is encoded by DQA1*0201 and DQB1*0202, and HLA-DQ8 by DQA1*0301 and DQB1*0302 [3, 20, 21]. More recent studies have identified over 100 non-HLA-related genes, linked to some degree of risk for developing CeD [13, 18]. HLA-DQ2/DQ8 are expressed on the surface of APCs and can initiate an immune response after binding with deamidated gluten. However, these variants are present in almost 40% of the world population but only a few develop CeD, which highlights the importance of environmental factors [3, 4]. Such influences under investigation are early-life infections, antibiotic and PPI use, feeding practices, and birth mode, all of which potentially could modulate the GM [4]. These factors alter the GM, but the exact way in which GM affects the CeD pathogenesis remains under study. Here are some recommended mechanisms:

- Gut bacteria share common epitopes with gliadin, allowing molecular mimicry and promoting immune activation (e.g. *Pseudomonas fluorescens*) [20];
- Lipopolysaccharides of Gram-negative bacteria cause the production of IL-15, which causes intestinal inflammation. IL-15 is believed to be a key cytokine in CeD, which antagonises the function of Treg cells that normally suppress the T-cell response [20];
- Toll-like receptor 3 in response to viral infections causes gut inflammation [14];
- The microbiota itself may cause the production of immunogenic gluten peptides, zonulin and proinflammatory or anti-inflammatory peptides [14, 18];
- Gut bacteria produce short-chain fatty acids (SCFAs), which act protectively to the intestinal permeability; loss of SCFAs may contribute to permeability disturbance [14];
- A decrease in microbiota species beneficial for gluten digestion (especially lactobacilli) has been also suggested as a potential mechanism [9];
- Finally, the metabolism of tryptophan (Trp) in the gastrointestinal tract produces the ligand of the aryl hydrocarbon receptor (AhR). AhR regulates the expression of genes like cyplal, IL22 and IL17 and through this it regulates immune responses at sites such as gut mucosa [22]. Lamas et al. [22] suggested that patients with active CeD exhibit impaired Trp metabolism by the gut microbiota and defective activation of the aryl hydrocarbon receptor (AhR), while metabolism by host immune cells is increased, leading to the production of proinflammatory kynurenine metabolites.

CeD and Environmental Factors

Antibiotics

Antibiotic use, especially in early life, affects the GM [10, 14, 18, 23]. Changes following a course of antibiotics may be lifelong [10]. However, studies on the relationship between antibiotic use and the pathogenesis of CeD show conflicting results [14]. A number of systematic reviews and meta-analyses have shown an increased risk of CeD with antibiotic exposure, possibly in a dose-dependent manner [10, 18, 24, 25]. It has been evidenced that in HLA-DQ8 positive mice, immunopathogenicity after gluten induction was influenced by antibiotic exposure [10]. Antibiotics may cause a decrease in *Bifidobacteria* species counts and an increase in *Bacteroides* species [18, 24]. Nevertheless, the evidence remains conflicting as studies in genetically at-risk children found no significant association between antibiotic use and either the development of CeD or CeD-specific autoantibodies [18]. Regarding to maternal exposure to antibiotics, available results are conflicting, with two studies on prenatal exposure to antibiotics (through the mother) showing significantly higher risk for developing CeD [10], while other investigators did not identify increased risk [18]. Conversely, antibiotics may sometimes be beneficial in cases of small intestine bacterial overgrowth (SIBO) related to poor response to a GFD [4].

Proton pumps inhibitors

Another factor possibly affecting GM is PPIs [3, 6, 10, 26]. The use of PPIs is believed to be associated with decreased bacterial richness and an unhealthy GM. This results in predisposition to *Clostridium difficile* infections [26]. Long-term use of PPIs can cause gastric bacterial overgrowth (e.g. *Streptococcus, Lactobacillus* and others), which leads to impaired bile acid creation, nausea and diarrhoea. A 2010 study found that 50% of PPI users had SIBO, while in non-users the proportion was 6%. In another study conducted in 2012 the proportions were 36% and 22% respectively [6]. In a population-based control study in 2014, CeD patients were more likely to have been prescribed PPIs before the diagnosis. A recent study has shown an association between PPI use and gut dysbiosis. However, the causal relationship between PPI use

and CeD development remains debated. Some studies suggest that PPI use may unmask underlying CeD symptoms, rather than directly increasing disease incidence [6]. There was also a significant increase of *Lactobacillus* spp. and *Streptococcus* spp., indicating a possible translocation of the bacteria from throat and oral cavities to the intestine[6].

Breastfeeding

In most studies, *breastfeeding* is shown to have a protective effect against CeD, through its impact on GM [4, 9, 15, 23, 24]. Breastfed infants have more *Clostridium leptum*, *Bifidobacterium longum* and *breve* than formula fed infants, who have more *Bacteroides fragilis, Clostridium coccoides - Eubacterium rectale* and *Echerichia coli* [9]. Infants fed with formula milk are more often colonised with *Echerichia coli, Bacteroides* and *Clostidioides difficile* compared with breastfed ones, while breastfed infants have a higher richness and diversity of *Bifidobacterium spp*. [27]. De Palma et al. [28] found that the type of feeding and the HLA genotype affected the gut colonization of the infants studied. HLA predisposition to CeD was associated with an increased number of Bacteroides fragilis and Staphylococcus and decreased *Bifidobacterium* [28]. These differences were more pronounced among formula-fed infants [15]. However, a number of studies do not confirm the protective effect of breastfeeding [15, 18].

Gastrointestinal infections

It is believed that there is a strong correlation between gastrointestinal viral exposure, especially in the first year of life, and CeD development, although the pathophysiologic mechanism remains unknown [7, 14, 18, 20, 23-25]. Toll-like receptors 3 are thought to play pivotal role [15]. Infection with enterovirus B during the first two years of life is believed to elevate the risk, especially when combined with a diet rich in gluten [7, 25]. Furthermore, vaccination against rotavirus seems to have a protective role against CeD, whereas rotavirus infection appears to increase the risk [7]. However, other studies have not found a consistent association between rotavirus infection and subsequent CeD development, suggesting that additional factors, such as genetic predisposition and timing of gluten introduction, may modify the risk [14, 18]. Other pathogens reported are adenovirus type 12, orthoreovirus and Candida albicans [14, 23]. More specifically, adenovirus type 12 has structural similarity to the amino acid sequence of gliadin; orthoreovirus induces inadequate immune stimulation, resulting in loss of tolerance to gliadin and increased inflammation and intestinal permeability. Candida albicans expresses a protein structurally similar to gliadin protein-1 [18, 23, 29].

Gluten introduction

There has been much concern about the impact of gluten introduction into an infant's diet. Studies show that delayed introduction at 12 months does not offer lower risk of CeD, but there is evidence that this may lead to late-onset disease [7]. On the other hand, in high risk infants, early introduction of small, fixed quantities of gluten has no effect on disease onset [7]. Finally, children diagnosed with CeD by the age of six had diets rich in gluten [7].

CELIAC DISEASE AND GUT MICROBIOTA CHANGES

Gut microbiota consists of several species of microorganisms, including bacteria, yeasts, and viruses. Bacteria are classified according to phyla, classes, orders, families, genera, and species. The two phyla *Firmicutes* and *Bacteroidetes* together represent approximately 90% of gut microbiota [13, 23, 27] The Firmicutes phylum is composed of more than 200 different genera, such as Lactobacillus, Bacillus, Clostridium, Enterococcus, and Ruminicoccus. Clostridium genera represent 95% of the Firmicutes phyla. Bacteroidetes consists of predominant genera, such as *Bacteroides* and *Prevotella* [13, 27, 29]. The *Actinobacteria phylum* is proportionally less abundant and mainly represented by the *Bifidobacterium genus* [27].

It is still debated whether dysbiosis plays a role in the pathogenesis of CeD or if it is a consequence of the disease itself [15]. The composition of gut bacteria is different in genetically predisposed children for CeD compared with low-risk children, even during the first years of life [23]. Many studies have tried to identify a gut microbiota pattern, associated with CeD pathogenesis or activity. However, their results show variation. Christofori et al. [15] reviewed 24 studies and concluded that, irrespective of the diverse results, there is an imbalance between proinflammatory and anti-inflammatory species, with prevalence of the former. According to available evidence, CeD patients seem to have microbiota with higher levels of *Bacteroids, E.coli, Proteobacteria* and *Staphylococcus* and lower levels of *Bifidobacteria* and *Lactobacillus* [15, 19, 27]. Some scattered studies have nevertheless shown that *Bifidobacteria* are associated with higher risk for CeD. This is probably explained by different *Bifidobacteria* species having different effects [1, 30].

Treated patients show a gut microbiota composition somewhere between active disease and healthy controls [9, 11, 31], but it may not be restored completely to a truly normal composition [32]. More specifically, while increased *Enterobacteria* or *Staphylococci* are restored, decreased *Bifidobacteria* and *Lactobacilli* and increased *Bacteroides*, *Enterobacteriaceae* and virulent *Echerichia coli* still persist [11, 15, 33]. On the other hand, GFD seems to alter the gut microbiota itself. There is a decrease of *Bifidobacterium*, *Clostridium lituseburense*, and *Faecalibacterium prausnitzii*, and an increase of *Enterobacteriaceae* and *Escherichia coli* counts. The reduction of beneficial gut populations could be due to the reduction in polysaccharide intake (fructans) while on GFD, as the latter has prebiotic action [15, 33]. Alterations in gut microbiota appear to play a role in the clinical manifestations of CeD. For instance, patients with gastrointestinal symptoms show altered microbiota composition (more *Proteobacteria phylum*). Patients with persistent gastrointestinal symptoms, although on strict GFD, show differences compared with the asymptomatic patients [15, 33]. *Pseudomonas aeruginosa* can enhance immunogenicity of 33-mer gliadin peptide, while other bacteria, such as *Lactobacilllus* and *Bifidobatceria* can decrease it, by facilitating gluten digestion [9, 23, 24]. Furthermore, oral microbiota plays an important role in gluten digestion and compared with healthy people, the saliva of CeD patients is rich in bacteria that could degrade gluten [34].

Whether dysbiosis is a cause or a result of CeD and how to modulate the balance of gut microbiota species for the benefit of CeD remains unclear and a focus of ongoing research.

THE USE OF PRO-, PRE- AND SYNBIOTICS IN CELIAC DISEASE

Although most evidence about the use of probiotics have been derived from animal models, this review focuses primarily on clinical studies investigating the effects of probiotics in CeD. Human studies, though still limited, provide the most relevant evidence for clinical practice [12, 16, 19]. Despite encouraging data from in vitro studies, there are very few in vivo data available, particularly in the paediatric population [12, 16, 19]. In the search for a microbial agent to modulate the disease, *Bifidobacteria* and *Lactobacilli* are the most extensively studied strains. Specifically, *Bifidobacteria* have been found to reduce the gluten induced epithelial permeability [23, 35] as well as to decrease Th1 pathway activation and reduce damage to the jejunal architecture [23]. Additionally, specific strains of *Lactobacilli* have demonstrated immunomodulatory characteristics in human studies, supporting their potential therapeutic use [23]. There is also evidence that, after consumption of lactobacilli-fermented wheat (gluten <10ppm) by CeD patients, there is no change in intestinal permeability nor an increase in IL2, IL10, and IFN levels in duodenal biopsies. This might be potentially useful for the reduction of toxicity in cases of incidental gluten exposure [3, 23, 25].

With regard to the clinical effects of probiotics, studies in which CeD patients received probiotics (*Bifidobacterium infantis, Bifidobacterium longum, Bifidobacterium breve*) showed improvement in gastrointestinal symptoms or greater increases in height percentiles compared with placebo [15]. Similarly, when CeD patients in remission were challenged for 2 months with *Lactobacilli*-predigested gluten, there was no exacerbating of symptoms, intestinal permeability or serological markers [9]. Animal studies, such as those involving *Saccharomyces boulardii* KK1, suggested a potential immunomodulatory effect, but clinical validation in human studies remains necessary [9, 13, 23]. Recent studies suggested that probiotic (especially multi-strain) intervention in CeD patients ameliorated gastrointestinal symptoms, including those of irritble bowel syndrome (IBS)-type [23, 33], decreased intestinal permeability and reduced pro-inflammatory cytokines [9, 12, 16, 23, 34]. A recent meta-analysis showed that probiotics were effective in improving gastrointestinal symptoms compared with placebo, but effect measurement was limited to the use of a specific questionnaire. No effect on inflammatory markers and intestinal permeability was found [17].

Prebiotics stimulate the growth and activity of beneficial bacterial strains, such as *Bifidobacterium* and *Lactobacillus*, and, as a result, can regulate the gut microbiota [23, 29, 35]. Consequently, the use of prebiotics along with GFD could be potentially beneficial in alleviating gastrointestinal symptoms or even by improving certain vitamins levels [23, 29, 36]. Only a few human studies have been conducted to date on the use of prebiotics, including inulin, lactulose and lactobionic acid, with promising results [23, 25, 35].

Concerning the use of synbiotics, a pilot study showed an improvement in gut barrier integrity, demonstrated by the increased expression of tight junction proteins in the group receiving the symbiotic [34]. However, larger and well-controlled trials are still needed to confirm these preliminary findings.

Postbiotics were only recently identified as another beneficial group of compounds. They are produced by probiotics when consuming prebiotics, are products of living bacteria or are generated after lysis. A well-known example corresponds to SCFAs. Postbiotics are believed to improve epithelial barrier function, modulate immune responses, and influence systemic metabolism and signaling via the nervous system. Compared to postbiotics are characterized by the advantages of having higher stability and safety, and that they are not living bacteria, so there is no risk of microbial infection or translocation [29, 34]. However, clinical data on the efficacy of postbiotics in CeD remains scarce, and further research is necessary.

All reviews conclude that despite encouraging data on probiotics use in CeD, the results are scarce, indefinite and heterogeneous across human studies. More studies are required to elucidate the underlying mechanisms and standardized protocols are required to accurately evaluate the use of pre-, pro-, syn- and postbiotics and their long-term effect [9, 13, 23, 34].

Fecal microbiota transplantation (FMT) is a procedure in which feces from a healthy donor are infused into a recipient's gastrointestinal tract to treat a gastrointestinal disease. Fecal microbiota transplantation was used in the past for the treatment of refractory *Clostridioides difficile* infection. Beurden et al. [37] reported a case of a 68-year-old patient with refractory CeD and *Clostridioides difficile* infection, who showed complete resolution of gastrointestinal symptoms and villous atrophy 6 months after FMT [37]. However, the use of FMT specifically for CeD remains experimental, with only isolated case reports available and no large-scale studies confirming its efficacy.

Limitations of Probiotics

Although probiotics are in mostly safe, there are some limitations. They may trigger allergic reactions, especially *Saccharomyces bulardii*, in patients with yeast allergy. Abdominal discomfort might develop during the first days of treatment, occasionally in conjunction with diarrhoea. Patients with central lines or impaired immunity are in risk for bacteriemia or infective endocarditis. Finally, some probiotics, such as enterococci, might transmit antibiotic resistance genes [26].

VIROME

The community of viruses in the gastrointestinal tract is called virome. Its biodiversity varies during life. The bacterial composition of the gastrointestinal tract and the virome are interdependent, and changes in the former can influence the latter. Viral dysbiosis has been reported in inflammatory bowel disease and CeD. Some phages have been proposed as potential prebiotics but the clinical application in CeD therapy is likely years away [29].

OSTEOPOROSIS

The aetiology of osteoporosis in CeD is believed to include multiple factors [5, 38]. A recent meta-analysis showed that 30-60% of newly diagnosed patients with CeD had low bone mineral density (BMD). There is a higher incidence of CeD in patients with osteoporosis (3.4%). Recently, the potential role of gut microbiota in bone health has gained attention, particularly through immune and metabolic pathways. The gut microbiota modulates the adaptive immune system, regulating nutrients absorption [36, 38-40] and bone turnover, through incretins, serotonin and IGF-1. Experimental studies have begun exploring how gut dysbiosis might influence bone physiology, although causal mechanisms remain unclear. A significant increase in trabecular bone density, thickness and mineral content has been shown when *Lactobacillus reuteri* ATCC 6475 was administered to healthy male mice. Additionally, a beneficial effect on rat bone health markers has been documented after administration of *Bifidobacterium longum, Lactobacillus rhamnosus* and *Lactobacillus paracasei* [38]. In humans, studies exploring gut microbiota and bone health in CeD are scarce, and do not yet support a robust clinical application. At present, no solid evidence supports that long-term microbiota modulation improves bone density outcomes in coeliac patients. Further high-quality clinical studies are required to investigate this potential "gut–bone axis" in the context of CeD.

CONCLUSIONS

Gut microbiota is a complex environment, composed of many different bacterial species. The interactions and balance between bacterial species are likely to impact the pathogenesis, treatment and course of many diseases, including CeD. Therefore, preventing or treating these diseases may require modulation of the gut microbiota to restore dysbiosis. Current evidence suggests that modulation of gut microbiota could be a promising strategy to prevent or treat CeD. Further research is required and should focus on: identifying pathological patterns of microbiota associated with specific diseases, such as CeD and to determine beneficial interventions using pro- pre- synbiotics; dosage and duration of treatment as well as evaluating long-term outcomes and safety.

Conflicts of interest: None to declare.

Authors' contributions: Conception/design L.M. and S.F. conceived and designed the study. L.M., S.F., D.M. and A.K. collected data. L.M., S.F.,M.P., K.D. analysed the data. L.M. drafted the manuscript. S.F., A.K., D.M., M.P. and K.D. revised the manuscript. All the authors read and approved the final version of the manuscript.

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