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Genetic and Environmental Contributors for Celiac Disease

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Abstract

Purpose of Review Celiac disease (CD) is an autoimmune enteropathy triggered by gluten. The purpose of this review is to examine the major genetic and environmental factors that contribute to CD pathogenesis.

Recent Findings We reviewed the current state of knowledge on the genetic and environmental components that play a role in CD onset. A genome-wide association study (GWAS) analysis has highlighted several genes other than HLA involved in CD. Recent studies have shown that HLA haplotype influences the microbiome composition in infants and that dysbiosis in the intestinal microflora, in turn, contributes to loss of tolerance to gluten. Recently, observational studies have discussed the hypothesis stating that breast-feeding had a protective role against CD onset.

Summary CD etiology is influenced by genetic and environmental factors. A better understanding of these components would deepen our knowledge on the mechanisms that lead to loss of tolerance and could help in developing a more "personalized medicine."

Keywords Celiac disease · HLA · Microbiome · Genetic · Environment · Gluten

Introduction

Celiac disease (CD) is a systemic, chronic, inflammatory small bowel, autoimmune disease standing as one of the few autoimmune diseases in which the environmental trigger, gluten, and the genetic predisposition, HLA DQ2 and/or DQ8, are well established [1, 2]. Gluten is a complex storage protein found in different grains and mainly composed of glutenin polymers and gliadin monomers that are not easily degraded in the gastrointestinal tract [3–5]. Presentation of CD has been noted in children as well as in adults and no specific age linked to the onset of disease has been reported [6]. It is estimated that 1% of the general population develops CD with a split ratio between female: male set at 2:1 [7]. In the USA, studies report CD frequency in adults and children as 1:105 and 1:322, respectively [6].

Although there is an increased rate of individuals affected by CD, the diagnostic process can still be challenging [8, 9]. Worldwide standardization of the clinical practice for diagnosing CD among physicians is recommended in that it would decrease the number of missed or delayed diagnoses and would therefore reduce serious complications and comorbidities such as type 1 diabetes, nutritional deficiencies, and neurological disorders [9].

Updated diagnosis guidelines include signs/symptoms compatible with the disease (with the exception of asymptomatic cases) and positive serological tests to be possibly confirmed by esophagogastroduodenoscopy showing intestinal enteropathy. Finally, the presence of the human leukocyte antigen (HLA) class II genes, part of the major histocompatibility complex (MHC) region, can also be used as additional diagnostic criteria for CD [3, 7, 10, 11], particularly if an endoscopy is not performed in accordance with the revised CD diagnostic criteria of the European Society of Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN).

The presence of intestinal enteropathy, ranging from an increase in intraepithelial lymphocytes (IELs) to villous atrophy and crypt hyperplasia, is a histopathological characteristic for CD diagnosis [6, 8].

The presences of elevated sensitive and specific serum antibodies, IgA-tissue transglutaminase (tTG) and anti-endomysium antibody (EMA), respectively, are used as initial

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and primary diagnostic markers [3]. In some cases, such as in that of IgA-deficiency, where tTG may not serve as an efficient diagnostic method, deamidated gliadin peptide (DGP) may also be tested avoiding false-negative reports [8, 9]. A 95% and an average of approximately 89% sensitivity for the screening tests, tTG and DGP, respectively, have been shown [1, 6, 7]. Studies have also shown a specificity of greater than 95% for EMA tests [3, 8, 9]. Recently, it has been reported that, in the case of tTG and EMA titers ten times greater than the limit and HLA compatibility, CD diagnosis can be determined without biopsy confirmation [8].

CD is a multisystem disorder causing classical gastrointestinal and extra-intestinal symptoms affecting skin (dermatitis), joints (osteoporosis), and other systems linked to peripheral neuropathy and headaches [8]. Children often presents with poor growth, anemia, and short stature among the classical gastrointestinal symptoms such as diarrhea [3, 10, 12]. Some individuals with CD, however, may also be completely asymptomatic [8].

Thus far, the only known and efficient treatment for CD to resolve the symptoms associated with the condition and normalizing the villous architecture is a strict, lifelong gluten-free diet [10].

Gluten: the Primary Environmental Trigger

Gluten is the recognized external trigger for CD. It disrupts barrier function and initiates an aberrant innate immune response characterized by high production of interleukin 15 (IL15), interleukin 8 (IL8), neutrophil recruitment, and expansion of intra-epithelial lymphocytes [13–16]. After translocating to the lamina propria, gluten peptides trigger a Th1/Th17-driven adaptive and humoral immune response further disrupting the barrier function and leading to intestinal damage [17].

The prevalence of CD is rising worldwide, along with a fivefold increase in gluten consumption and a substantial change in gluten's protein contents [3, 18]. Some research has suggested that the reported increased gluten consumption may contribute to the rise of CD rate [19, 20]. Given the lack of sufficient data on yearly CD incidence, however, it is impossible to determine with confidence if the pro-capita intake of gluten may actually influence CD epidemiology.

The importance of early feeding practices regarding food allergies and intolerances is well recognized [21]. Observational studies have suggested that the time of gluten introduction in infants' diet may represent a risk factor for CD [19, 22]. Following these findings, in 2008, the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) recommended the introduction of gluten between 4 and 7 months [23]. In contrast with these data, two randomized control trials (PREVENTCD and CELIPREV) have showed no correlation between the CD onset and time of gluten introduction [24••, 25••]. The same studies have also reported that gluten ingestion after 12 months of age may delay the development of the disease without totally preventing it.

In addition to the time of gluten introduction, the amount of ingested gluten has also been proposed as a risk factor for CD in children under 2 years of age, while the same effect was not reported in older kids [19]. Although interesting, the results of these studies did not take into account genetic differences among patients and have not been confirmed by more recent interventional studies [25••]. Given its fundamental role as environmental trigger for CD, gluten has long been considered a target for preventive therapies such as detoxification of gluten proteins with enzymatic methods [26, 27] and grain genomic modification [28, 29]. Although promising, to this day, gluten-modifying therapies have not yet been shown to be efficient in dramatically reducing gluten toxicity [30], and more research is needed to investigate their potential.

Genetics

CD is a unique model of autoimmunity for which the genetic background necessary for the development of the disease is well known. Specific HLA genes are considered necessary but not sufficient to lose tolerance to gluten, and more recently, other genes have been involved in CD pathogenesis as well (Table 1).

HLA Genes

CD pathogenesis involves the adaptive and innate immune systems. Once gluten is ingested, the protein peptides gain access to the intestinal lamina propria [6]; they are deamidated, recognized by antigen-presenting cells (APCs) through HLA class II molecules; and finally trigger an aberrant $CD4^+$ T cell–mediated immune reaction [8]. Creation of peptide-HLA complex on APCs is responsible for the transcription, configuration, and signaling preferences for the events involved in CD, thus being an essential step of CD pathogenesis [6, 11, 31].

HLA molecules are composed of two subunits, the alpha and the beta subunits [6, 32, 33]. They are part of the MHC region and they account for 40% of the genetic CD variance.

The disease-associated HLA class II genes, DQA1*05 and DQB1*02 encoded in DQ2.5 heterodimer, the higher risk heterodimer, and DQA1*03 and DQB1*03 encoded in DQ8 heterodimer, are located on chromosome 6p21 [7, 31, 34].

The strength of immunological responses is given by the DQA1*05 and DQB1*02 gene dosage on APCs. Studies have shown the majority of people affected by CD have the HLA DQ2.5 gene, proving those who have this gene have a higher risk for CD development [8, 11, 31]. Further risk is observed if

Table 1 Prominent genetic factors linked to the pathogenesis of celiac disease

Genetic factor	Candidate genes	Role in pathogenesis of disease
MHC genes (HLA)	• HLA class I: DQB*0801 • HLA class II: DQA1*05; DQB1*02; DQA1*03; DQB1*03	• Peptide-specific recognition [31] • T cell presentation/reactivity [7, 31]
Non-MHC (non-HLA) genes	• IL2; IL21 • IL18R1; IL18RAP MYO9B, HP2, PARD3, MAGI2	• B and T cell activation and proliferation [7] • Role in pro-inflammatory signaling cascade [7] • Intestinal permeability regulation [41]

the HLA-DQ2.5 heterodimer is in a homozygotic configuration rather than in heterozygotic [35].

Twin and family studies indicate genetics playing a critical role in CD development [35, 36]. Interestingly, there are studies also demonstrating that HLA DQ2 haplotypes are associated with early onset of disease while HLA DQ8 haplotypes are associated with onset of disease in adults rather than in children [37]. Although insufficient and costly, HLA class II genes are the strongest risk markers for CD development [11, 38].

Non-HLA Genes

Advances in genetic research shows that the genetic contributors to CD are split between the MHC and non-MHC region genes [7, 31]. The immunologic responses seen in CD is the result of many cell functions, so it is important to acknowledge and deepen the knowledge of the genetic factors associated with CD. Genome-wide association studies (GWAS) have found an 18% genetic heritability, listing 18 other risk factors in the MHC region aside from the HLA class II genes that play a role in CD. They describe HLA class I genes as a role player in CD pathogenesis and in other immune-mediated diseases [31]. GWAS studies additionally linked 39 non-HLA genes [8] associated with the descriptive, diagnostic features of CD. These loci encode for genes that contribute to different steps of the CD pathogenesis (altered gut barrier function and aberrant immune response) [7, 31]. To name a few, two genes involved in T cell maturation and differentiation, *IL2* and *IL21*, have shown high association with CD [39] and are considered strong candidate genes. *IL18R1* and *IL18RAP* are fundamental for the pro-inflammatory immune response and have been shown to be highly expressed in CD patients independently on the disease state (remission or active) [39]. Similarly, other immune-related genes encoding for chemokines and involved in B cell proliferation have been found to influence CD susceptibility [39]. *MYO9B* (myosin IX B) and *HP2* (haptoglobin2), *PARD3* (par-3 family cell polarity regulator), and *MAGI2* (membrane-associated guanylate kinase2) genes are involved in intestinal permeability regulation and have been associated with a higher risk of developing CD [39, 40]. Regions 2p16 and 6q23 on chromosome 4 are newly identified regions encoding genes responsible for functional properties of interleukins and prominent signaling pathways that could contribute to CD development [7, 32]. In a recent work, Leonard et al. used whole genome sequencing analysis on duodenal biopsies to investigate the transcriptional profile in CD. The authors found that, in addition to genes that have been previously correlated with CD, the active state of the disease, in which enteropathy was present, was also characterized by dysregulation of genes associated with spliceosome formation, cell cycle processes, and other immune functions [41••].

This newly found heritability beyond the MHC region is reported in different populations in Europe and in the USA [7]. Further research studying these newly found loci, HLA and non-HLA, and the interplay between genetics and environmental factors may be the key providing further methods of intervention and/or treatment for immune-mediated disease beyond CD.

Other Prominent Environmental Factors

It is now well accepted that additional environmental factors other than gluten strongly characterize CD etiology [1]. This is corroborated by the rapid number of increased CD diagnoses, especially among adult patients [42], and by the rising incidence of refractory CD, where affected individuals are irresponsive to the gluten-free diet [43]. Below we investigate some of the main "candidates" reported in literature (Table 2).

Breast-feeding

For several years, breast-feeding has been hypothesized to have a protective role against CD onset through different mechanisms [44]. Observational studies have reported that increased duration of breast-feeding correlates with reduced risk of developing CD. Similarly, in 2013, Ivarsson et al. showed that gluten introduction while breast-feeding significantly reduces the risk of CD in children under 2 years of age [19]. These results, however, were not confirmed by later studies [24••, 45].

Table 2 Main environmental factors that have been proposed to influence celiac disease development

Environmental factor	Suggested effect	Proposed mechanism
Time and amount of gluten introduction in infants' diet	Aberrant immune response triggered by toxic, chemotactic, and immunogenic peptides	• Early introduction of considerable amount gluten in infants has been suggested to trigger CD [19, 20]. Data not confirmed by observational studies [24, 25]
Breast-feeding	Protection against CD development and/or delay of its onset	• IgA-mediated immune modulation [44] • Indirect effect on amount/time of gluten and cow's milk ingested by infants [45] • Changes in infants microbiome [46]
Infections	Protective or triggering effect on CD development	• Attenuation of host immune response [60] • Reduction of antigen immunogenicity [60] • Changes in microbiome composition [61] • Modulation of intestinal permeability [51]
Microbiome	Contribution to loss of tolerance to gluten	• Immune response regulation [86] • Barrier function modulation [92] • Changes in gluten peptide immunogenic properties [100]

Breast-feeding has been hypothesized to play a role in CD onset through five different means: (1) the presence of gluten-specific IgA antibodies and immune system modulators might influence tolerance induction [44], (2) children who are still breast-fed are more likely to ingest less amounts of gluten [45], (3) longer breast-feeding habits may delay infants' encounter with cow's milk protein [46], and (4) human breast milk has a known effect on modulating infants' microbiome composition [47].

Despite the lack of strong evidence demonstrating a protective role of breast-feeding against CD, its overall health benefits are well established. Further studies are deemed to investigate more mechanistically if and how the maternal milk modulates the infants' tolerance to gluten.

Infections

Repeated gastrointestinal infections have been hypothesized to increase the risk of CD [22]. Infections may cause intestinal inflammation, therefore reducing tolerance to gluten in genetically predisposed individuals [48]. Furthermore, exposure to specific bacteria or viruses can trigger the activation of polyclonal lymphocytes, antigen molecular mimicry, and increased immune response against auto-antigens following infection-driven inflammation [49, 50]. Finally, gastrointestinal infections can also shape the microbiome composition of the host [51••]. All of these mechanisms may alter the immune response ultimately contributing to CD onset [22].

While some studies have suggested that perinatal infections may influence CD outcome [49], most of the research has been focusing on the effect neonatal infections can have on genetically predisposed individuals [52]. This is not surprising given that the infants' gastrointestinal tract is immature and, therefore, particularly vulnerable and prone to changes in regard to microbiome composition and immune tolerance establishment [51••]. Different kinds of pathogens have been associated with the risk of CD. Infections from *Campylobacter jejuni* [53], *Toxoplasma gondii* [54], *Giardia lamblia* [55], hepatitis C virus [56], and rotavirus [57] have all been correlated with CD development. These associations, however, were mostly based on single-case studies or could not have been concluded as casual [45]. Interestingly, some pathogens have been hypothesized to have a protective role against CD onset [58]. Jansen et al. reported a single-case study in which single *Cytomegalovirus* (CMV) infection, or combined with Epstein-Barr virus (EBV), was inversely correlated with tTG-IgA antibodies [51••]. The protective role of EBV infection against CD remains under debate. The hypothesis was based on the low levels of antibodies anti-EBV found in CD patients [58], which may be explained by the fact that these specific antibodies are usually produced within the first week of the infection and do not always reflect the presence of virus' DNA [51••]. Similar considerations can be made about the suggested protective role of *Herpesvirus* and *Rubella* infections [51••]. In addition, *Helicobacter pylori* has been proposed as an additional "beneficial bug" against CD development, although the data collected to this day are not conclusive, showing only a minimal protective effect of the bacterium in the adult population [59]. Bacterial and viral infections have been suggested to decrease the risk of CD through different mechanisms: (1) microbial enzymes may reduce immunogenicity of specific antigens via post-translational modifications [60], (2) pathogens can alter host microflora due to competition for nutrients [61], (3) some pathogens modulate intestinal permeability therefore counteracting the disruption of the tight junctions that characterizes CD pathogenesis [51••], and finally (4) different microbes and helminths can attenuate the host immune response through molecular mimicry with self-antigens [62] by triggering a shift between Th1 and Th2 immune response [63], secreting anti-autoreactive T cell proteins [64] or extracellular vesicles [65].

The relation between pathogens and CD is complex and needs to be further investigated. A better understanding of the contribution of infections to CD pathogenesis is necessary in

that it would highlight potential new use of probiotics as well as identification of at higher-risk populations.

Microbiome

The human gastrointestinal tract harbors an extremely diverse microflora composed of over 10^{18} bacterial cells [66]. Novel culture-independent approaches allowing the categorization of this microbial community in specific taxonomic groups [67] and in vivo experiments using germ-free mice have highlighted the fundamental role that the microbiome plays in inducing and maintaining the intestinal homeostasis [68].

Gut-colonizing bacteria contribute to the harvest of nutrients and energy that are fundamental in carbohydrates' fermentation and protecting the host from pathogens by competing for the same nutrients [69]. In addition, they are also instrumental in shaping the local mucosal gut-associated lymphoid tissue by driving T-effector cell differentiation [70], modulating immune cell response [71], and regulating the epithelial barrier function [72, 73].

Given the numerous tasks that commensal bacteria perform to modulate the intestinal homeostasis [74], the correlation between dysbiosis and different diseases such as type 1 diabetes [75], obesity [76], IBD [77], and autism [78] is unsurprising.

Like other autoimmune diseases, CD has been associated with alterations of the microbiome's composition in both pediatric and adult populations [79]. Studies on duodenal biopsies and fecal samples have shown an increased abundance of *Bacteroidetes* [80] and *Proteobacteria phyla* [53], as well as higher frequency of *Clostridium* [81], *Bacteroides* [80], and *Prevotella* spp. [82], in active CD patients. *Lactobacillus* and *Bifidobacterium* spp. abundances, on the contrary, appear decreased when compared with controls [83]. Furthermore, these changes in the microbiome have been correlated with alterations in specific metabolites' production [83] and with the clinical manifestations of the disease [84].

Although informative, the abovementioned studies have correlated intestinal dysbiosis only with the acute phase of CD without examining the causality between the two conditions. In recent years, researchers have begun investigating whether alterations in the microbiome composition may actively contribute to the loss of tolerance to gluten. In fact, the microflora of an infant is extremely plastic and can be strongly modulated by external factors such as delivery mode, feeding habits, use of probiotics/antibiotics, maternal microbiome, and genetic background [85]. In their proof of concept study, Sellitto et al. recruited infants genetically at risk for CD and followed them prospectively until 2 years of age [86]. 16s gene analysis demonstrated that compared with the low-risk subjects, infants carrying the CD-predisposing HLA haplotype had increased *Firmicutes* and *Proteobacteria*, while *Bacteroidetes* and *Actinobacteria* were significantly reduced. Interestingly, these patterns persisted up to 2 years of age and correlated with alterations in microbial-derived metabolite production [86]. Additional confirmation of dysbiosis preceding the onset of the disease has also recently been described, by Olivares et al., reporting a decreased abundance of *Bifidobacterium longum*, and, a higher frequency of *Bifidobacterium breve* and *Enterococcus* spp. in at risk infants [87••].

The hypothesis that a specific genetic background may contribute to CD development by triggering changes in the microbiome has been supported by several studies underlining the effect that HLA DQ genotype has in regulating the gut colonization process [88, 89].

By highlighting alterations in the microbiome composition prior to the onset of CD, these studies strongly suggest that dysbiosis can be considered an additional environmental contributor to the loss of tolerance to gluten rather than only a direct consequence of the chronic enteropathy.

The hypothesis that the microbiome may represent a contributing factor to CD pathogenesis is also strengthened by the increasing number of diagnoses among adult patients [12]. Although the microbiome stabilizes at around 2–3 years of age [68], it is still considered an extremely plastic entity that can change its composition during adulthood under the influence of external elements including antibiotic use, stress, nutrition, and infections [90]. Alterations in the microbiota, therefore, could be accounted for sudden loss of tolerance to gluten later in life independently of the timing of gluten introduction [91].

Mechanistic links between dysbiosis and CD development have been suggested by studies showing the capability of microbial bioproducts to modulate the regulatory immune system and the intestinal epithelium. Altered production of butyrate and lactate has been shown to trigger post-translational modifications in T regulatory cells in the intestine of CD patients, interfering with their suppressive role [92]. Furthermore, Freire et al. have demonstrated that these metabolites modulate the intestinal barrier function and the inflammatory response triggered by gliadin [93].

Numerous groups have suggested that the microbiome may also contribute to CD development through its proteolytic properties. Caminero et al. have reported that *P. aeruginosa*, found in CD patients, cleaves gliadin peptides whose immunogenicity can be reduced by *Lactobacilli* spp. found in non-CD controls [94]. An increased proteolytic activity against gluten peptides that correlates with *Proteobacteria's* abundance has been shown in CD patients [95], while *Lactobacillus helveticus* has been reported to efficiently cleave numerous immunogenetic gliadin peptides [95]. Similarly, a recent study using in vivo HLA $DQ8^{+}$ mice has demonstrated *Lactobacilli* reducing intestinal inflammation by degrading wheat amylase trypsin inhibitors [96].

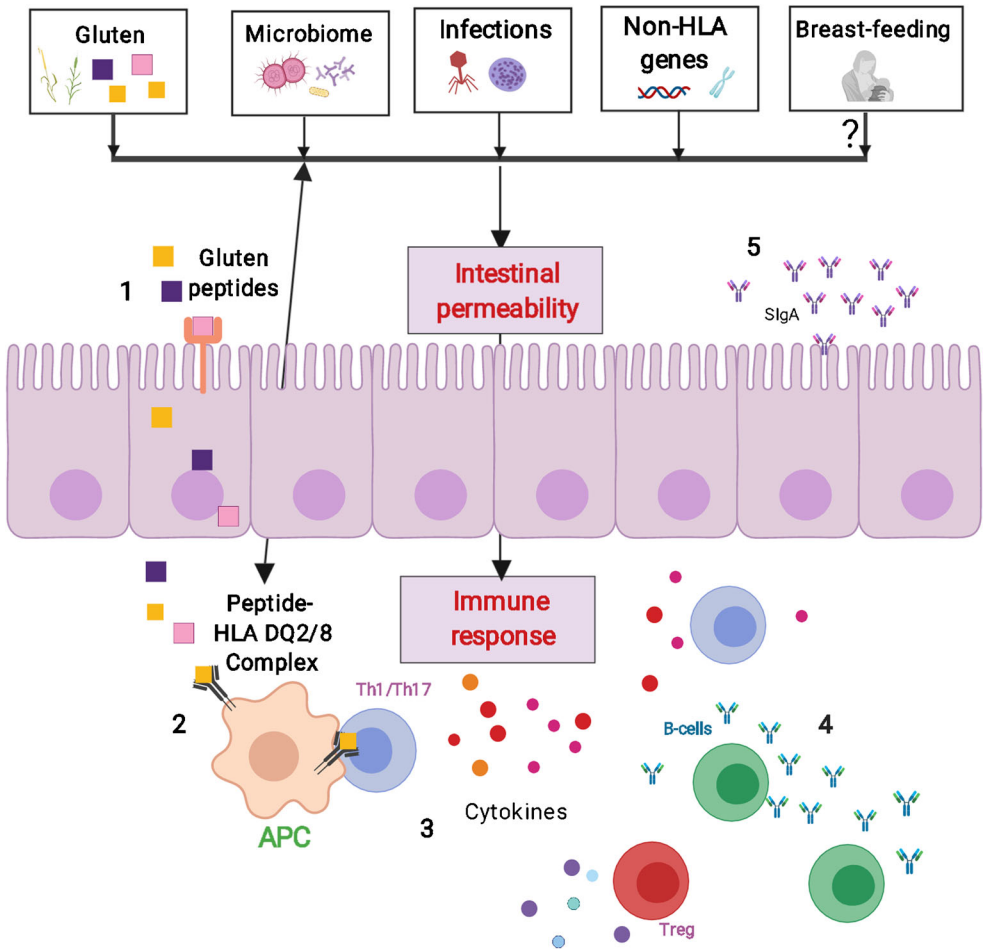

Fig. 1 Schematic overview of environmental and genetic contribution to certain events in celiac disease pathogenesis. Environmental and genetic factors are proposed as contributors to celiac disease by influencing intestinal permeability and overall immune response. In patients with celiac disease, partially digested gluten peptides trigger an increased intestinal permeability [1] that can be modulated by commensal bacteria, infections, gluten composition, and non-HLA genes. This allows the translocation of the peptides to the lamina propria where they are recognized by specific HLA DQ2/DQ8 [2]. The initiation of an inflammatory immune response with TH1/TH17 cells [3] can be differentially regulated by infections, commensal bacteria, gluten composition, and non-HLA genes. Similarly, the following humoral immune response [4] and release of secretory IgA [5] can be influenced by these environmental and genetic factors. This figure has been created with Biorender Software

In addition to the small intestinal dysbiosis, salivary microflora, whose role is hydrolyzing proline and glutamine-rich peptides, has also been shown to be altered in CD patients [97].

Aberrant microbiome has also been found in the blood of adult subjects affected by CD [98]. While the function of the blood microbiome remains under debate, in the last decade, several studies have shown associations between blood dysbiosis and different diseases [98]. In a recent study, hematic microbiome of CD patients has been characterized by a reduction of *Clostridiales* and increased *Bacillales*. These changes appeared to be independent of the disease status, thereby refuting the hypothesis linking them exclusively to the intestinal enteropathy. The concept of blood microbiome is still under debate; in fact, the sole detection of bacterial DNA does not confirm the presence of viable microorganisms in the blood. While further studies are needed to investigate the function and characteristics of the hematic microflora, the differences found between CD patients and healthy controls are intriguing and support the hypothesis that changes in the microbiome may play a role in priming circulating immune cells towards a less tolerant phenotype in CD patients [92].

Given the pathogenic implications that intestinal dysbiosis can have on the host, the use of probiotics has been suggested as a supplemental treatment for refractory CD patients [31]. Specific *Lactobacilli* strains have been shown to reduce the immunotoxicity of gluten [99], and, probiotic cocktail VSL#3 and *Lactobacillus rhamnosus* have been shown to ameliorate intestinal barrier function [100]. Similarly, recent studies on

Bifidobacterium breve and *longum* have described the anti-inflammatory properties of these bacteria on children with CD [101]. While many clinical trials involving the use of probiotic to treat and prevent CD [30] are promising, more prospective studies on the matter are deemed to better understand the exact mechanisms linking dysbiosis and probiotics with the onset and development of the disease.

Conclusions

CD is a unique example of autoimmune disease, in that the external trigger, gluten, and the genetic background necessary for its development are well known. The last few decades, however, have been characterized by a rising incidence of CD. While part of this phenomenon can be related to better diagnostic tools and awareness of the disease, it is also suggested that additional genetic and environmental components may contribute to the disease development. GWAS and, more recently, whole genome sequencing and RNAseq studies have highlighted numerous genes, other than HLA, that may be involved in CD pathogenesis (Fig. 1). Mutations in the genetic background cannot fully explain the rapid increase in CD diagnosis; however, a better understanding of the genes involved in CD pathogenesis can give important insights about the mechanisms regulating the loss of tolerance to gluten. Furthermore, a targeted genetic screening among at-risk infants may help with early diagnoses, patient stratification, and prevention of comorbidities onset.

Besides the genetic component, one major factor contributing to CD onset is the environment (Fig. 1). Changes in the environment could explain the rapidity with which CD incidence rose in line with other autoimmune diseases. A better understanding of the environmental factors that play a role in CD onset and progression is fundamental to deepen our knowledge on CD pathogenesis, but also to research new therapeutic and preventive strategies.

Further prospective and longitudinal studies are needed to investigate in detail the different components that may influence CD onset with the final goal of introducing personalized medicine.

Compliance with Ethical Standards

Conflict of Interest The authors declare no conflicts of interest relevant to this manuscript.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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