Celiac Disease and the Microbiome

Francesco Valitutti 1,2,*, Salvatore Cucchiara 1 and Alessio Fasano 2,3,4

1 Pediatric Gastroenterology and Liver Unit, Department of “Maternal-and-Child Health” and Urology, Sapienza University of Rome, 00185 Rome, Italy; salvatore.cucchiara@uniroma1.it
2 European Biomedical Research Institute of Salerno, 84125 Salerno, Italy; AFASANO@mgh.harvard.edu
3 Center for Celiac Research and Treatment, Mucosal Immunology and Biology Research Center, Mass General Hospital for Children, Boston, MA 02114, USA
4 Division of Pediatric Gastroenterology and Nutrition, Mass General Hospital for Children, Boston, MA 02114, USA
* Correspondence: francesco.valitutti@gmail.com

Received: 17 August 2019; Accepted: 27 September 2019; Published: 8 October 2019

Abstract: Growing evidence supports the hypothesis that changes in both the composition and function of the intestinal microbiome are associated with a number of chronic inflammatory diseases including celiac disease (CD). One of the major advances in the field of microbiome studies over the last few decades has been the development of culture-independent approaches to identify and quantify the components of the human microbiota. The study of nucleic acids DNA and RNA found in feces or other biological samples bypasses the need for tissue cultures and also allows the characterization of non-cultivable microbes. Current evidence on the composition of the intestinal microbiome and its role as a causative trigger for CD is highly heterogeneous and sometimes contradictory. This review is aimed at summarizing both pre-clinical (basic science data) and clinical (cross-sectional and prospective studies) evidence addressing the relationship between the intestinal microbiome and CD.

Keywords: celiac disease; microbiome; microbiota; environmental factors; at-risk infants

1. Introduction

Although the recognition of the causal link between gluten and celiac disease (CD) was unveiled in the 1950s [1], what factor (or factors) triggers the loss of immune tolerance to gluten in genetically predisposed subjects still remains unknown. Since its original description, CD has most often been perceived as a pediatric condition with a peak incidence in children younger than two years of age, with more recent data suggesting that most of the cases would manifest by five years of age [2].

The worldwide prevalence of CD ranges between 1% and 2% in the general population [3,4], with most patients remaining undiagnosed due to the subtle or multiform clinical manifestations of the disease [5]. Based on more recent epidemiological data, and contrary to the original paradigm, it is now appreciated that CD can present at any age with a broad range of intestinal and extra-intestinal symptoms [6,7]. Its prevalence, as in many other autoimmune diseases often found in comorbidity with CD [8], has increased over time in geographical regions characterized by a Western lifestyle [9]. This phenomenon was initially hypothesized to be secondary to the timing of gluten introduction at weaning [10], although two large, randomized, and prospective high-risk, birth cohort-controlled trials have disputed this premise by demonstrating that neither delayed nor early gluten introduction modified the risk of CD [2,11].

These findings raised doubts about another CD paradigm that suggested that genetic background and dietary gluten intake were necessary and sufficient to develop the disease. Besides the evidence that CD onset can occur years after gluten introduction into the diet [6], other evidence at odds with
the old paradigm is the lack of 100% CD concordance among monozygotic twins [12]. Therefore, while genetic predisposition (including the required presence of HLA DQ2 and/or DQ8 haplotypes) and gluten exposure are necessary, they seem to be insufficient for the development of CD autoimmunity. Intestinal permeability is an additional element involved in CD pathogenesis, as a “leaky gut” might initiate the early phases of innate immune activation following the exaggerated trafficking of undigested gluten fragments from the intestinal lumen to the lamina propria [13].

Growing evidence supports the hypothesis that changes in gut microbiome composition and function are associated with a number of chronic inflammatory diseases including obesity [14], diabetes [15], inflammatory bowel disease [16] and cancer [17]. This might also be the case for CD.

In the last decades, one of the major advances in the field of microbiome studies has been the ability to apply culture-independent approaches to determine the microbiome’s composition [18]. These technologies allow for the identification and quantification of components of the human microbiota by studying nucleic acids (DNA and RNA) from fecal samples or other biological samples [19], which eliminates the need for tissue cultures and also allows the characterization of non-cultivable microbes.

The human gastrointestinal lumen contains a copious and diverse microbial ecosystem of over 100 trillion microorganisms [20]. More than 2 million genes are expressed by the human microbiome, and these genes encode for metabolic pathways that finally produce thousands of metabolites [21]. Conversely, it is striking to note that the human genome is composed of only 23,000 genes [22]. Consequently, the host and its microbial communities can be viewed as a “superorganism” with mutable immune and metabolic profiles [23].

Gut bacteria facilitate the digestion of insoluble fiber, produce vitamins such as vitamin K, and elaborate trophic and immunomodulating compounds such as short-chain fatty acids (SCFA) [24].

Moreover, they also display key immune-modulating functions within the gut. By competing for nutritional sources and producing anti-microbial molecules, beneficial gut bacteria counterbalance the growth of pathogenic bacteria and favor epithelial integrity [25,26]. Microbiome-derived SCFA can also modulate host histone deacetylase, therefore epigenetically influencing the function of innate and adaptive immune cells [27]. The impact of the gut microbiome on mucosal immunity is further demonstrated by the evidence of defects in lymphoid tissues (a decreased number of mucosal Peyer’s patches and smaller mesenteric lymph nodes) and compromised antibody production in germ-free animals [28].

In early childhood, microbial diversity rises with age until it stabilizes with two major bacterial phyla: Firmicutes and Bacteroidetes, which represent roughly 90% of the whole gut microbiota. Actinobacteria, Proteobacteria, Fusobacteria and Verrucomicrobia are the next most-numerous describing a “healthy gut microbiota composition” [29]. At approximately three years of age, a child’s gut microbiota composition and diversity are very similar to the adult microbiota [30]. While it is generally assumed that microbiome engraftment occurs at birth during the passage through the vaginal canal, or via maternal skin microbiota in case of cesarean section, there are a few reports showing that a specific microbiota colonizes the placenta [31] and is detectable in the meconium [32], suggesting that engraftment may start in utero.

In recent years, research into the early development of the microbiome has highlighted the influences of delivery mode, maternal/infant nutrition and antibiotics on the engraftment and subsequent changes in intestinal microbiome composition [33,34]. This crucial initial symbiotic relationship between host and gut microbiome is instrumental in programming the immune system to distinguish between pathogens and commensals to achieve the proper strategies to unleash inflammation when necessary (for example fighting pathogens) or maintain anergy [35].

This review is aimed at summarizing current evidence on the relationship between the gut microbiome and CD. For the sake of brevity, no studies on the microbiomes of patients on a gluten-free diet (GFD) have been considered. This choice is also justified by the fact that gluten dietary exclusion
would represent an “intervention” affecting gut microbiome composition, thus introducing a strong bias for further considerations.

The literature search was run using Pubmed, EMBASE, Web of Science and Scopus using terms as: “microbiome and CD” (341 articles), “microbiota and CD” (301 articles), “gut microbiome and CD” (152 articles) and “gut microbiota and CD” (220 articles). The search was limited to articles written in English. All abstract papers were read, 153 were analyzed as full articles, and finally, only 129 were included as references for this review.

2. Microbiome, Environmental Factors and Gut Inflammation: Implications for Celiac Disease (CD)

Environmental factors strongly drive microbiota engraftment and subsequent composition. For example, vaginal delivery ensures the vertical mother–infant transmission for pivotal gut microbiome components such as *Bacteroides* and *Bifidobacteria* [36]. Conversely, cesarean (C) section-born infants show less *Bacteroidetes*, and the diversity of this specific phylum is lower [37]. However, while it is uncertain if these changes might explain some reports of an increased risk of CD for children born via C-section [38,39], it should be acknowledged that the association between C-section and CD is still controversial [40].

Diet is another key regulator of microbiome development and homeostasis. The human milk oligosaccharides (HMOs) select the growth of commensals such as *Bifidobacteria* and prevent the growth of potential pathogens such as *Clostridium difficile* [41,42]. Moreover, HMOs enhance overall barrier integrity by making enterocytes less vulnerable to bacterial-induced innate immunity [43]. Therefore, breast-feeding seems to be ideal for the engraftment of a symbiotic gut microbiome.

Some data also suggested that maternal antibiotic assumption during pregnancy shapes the gut microbiota in the offspring [44], albeit a cohort study found no statistically significant association between maternal use of antibiotics during pregnancy and CD risk in the offspring [45]. According to some reports, antibiotic exposure during the first year of life has been associated with an increased risk of developing CD [46,47], however, other studies did not confirm this finding [48–50]. A recent meta-analysis did not resolve these incongruences, albeit favoring a non-causal relationship between early antibiotics exposure and CD [51].

Early life infections may be involved in CD onset, and this issue is also supported by cohort studies [52,53]. Another study that looked at the effect of viral triggers and Th1 response recognized reovirus as a possible cofactor for both inappropriate immune activation and subsequent loss of tolerance to gliadin [54]. Patients with CD display higher antibody titers against human adenovirus serotype 2 [55,56]. This might go along with the clinical interpretation of in vivo data. A longitudinal prospective cohort of genetically at-risk children demonstrated that an increased rate of rotavirus gastroenteritis may strengthen the risk of CD in infancy [57]. However, the implementation of rotavirus vaccination did not prevent a rise in CD prevalence that has been recently reported in Italian children [58]. A role for *Candida albicans* in CD development has been hypothesized based on sequence similarities between a hyphal wall protein and several T-cell gliadin epitopes [59], albeit the only small study on mycobione next-generation sequencing analysis of duodenal samples showed no difference between adult CD cases and controls [60]. A large cohort study from Sweden has shown that there is a significantly higher hazard ratio of *C. difficile* infection in patients with CD when compared to age- and gender-matched controls [61], albeit study limitations leave open a few areas of uncertainties [62].

The physical isolation of microbes from the glycolax of the intestinal epithelium without evidence of overt inflammation suggests that preventing physical contact with the gut mucosa avoids activation of the immune system, therefore favoring a symbiotic relationship between the host and the gut microbiome [63]. A balanced gut microbiota also contributes to the maintenance of the mucosal layer, especially due to bacteria such as *Lactobacillus* species and *Akkermansia muciniphila* [64,65]. A healthy microbiota additionally favors colonization resistance, namely, the capability of commensal bacteria to compete for nutrients with pathogens, thereby stimulating the epithelium to secrete...
antimicrobial molecules into the mucous layer and provide a better defense against pathogens [66]. Additionally, commensals contribute to this line of defense by synthesizing protective substances, such as acetate produced by Bifidobacterium, which prevents colonization by enterohemorrhagic E. coli O157:H7 [67]. IgAs produced by the gut-associated lymphoid tissue (GALT) also contribute to barrier maintenance, microbiome selection and decreased activation of innate immunity [68]. Related to this topic, Olivares et al. demonstrated that a reduction in IgA fecal level can precede CD development in infants [69].

Some studies have shown an increased expression of genes responsible for pathogen-associated molecular pattern (PAMPs) recognition, such as Toll-like receptors (TLR) in CD. For example, Szébeni et al. found higher expressions of TLR2 and TLR4 in untreated and treated CD patients versus controls, as shown both at mRNA and protein levels [70]. Furthermore, TLR2, TLR9 and TOLLIP, an intracellular protein that inhibits TLR, have been found as microbiota-associated factors in the possible development of CD [71]. Overexpression of TOLLIP in vitro offsets TLR pathways after lipopolysaccharide or lipoteichoic acid stimulation. This phenomenon has been named “lipopolysaccharide tolerance” [72]. In fact, a reduced expression of TOLLIP in active CD might indicate that a failure to tolerate microbiota may contribute to CD immune activation.

It is well acknowledged that the host can tightly control the microbiota, however, the microbiota also exerts a strong programming on host metabolism and immunity [73]. SCFA synthesized by commensal bacteria condition regulatory T-cells (Treg cells), specifically, one member of the SCFA, butyrate, helps T-cells to differentiate toward Treg cells [74]. SCFA might inhibit histone deacetylases, provoking hyperacetylation of histones, which finally results in anti-inflammatory gene activation [75].

The role of gut microbiota and their metabolites in CD has been explored by a recent study showing their effects on Treg cells through epigenetic processes [76]. Specifically, CD patients showed an increased expression of a non-functional spliced form of FOXP3 (so increasing the risk of developing autoimmunity) which could be attributable to the altered intestinal microbiota and to its unbalanced butyrate production.

In another study, CD-derived organoids treated for 48 h with microbiota-derived compounds, such as lactate, butyrate and polysaccharide A, showed a significant improvement of intestinal permeability measured as transepithelial electrical resistance changes. Moreover, the same group also showed that butyrate significantly upregulated the expression of genes regulating epithelial integrity in CD organoids [77].

It has been noticed that the HLA-DQ genotype can affect early gut microbiota composition [78], and an increased occurrence of pathogenic bacteria such as enterotoxigenic Escherichia coli has also been described in infants genetically at risk for CD [79]. In a previous study from a Spanish group, higher numbers of Bifidobacterium spp. and Bifidobacterium longum were present in the gut microbiota of infants with the lowest HLA-DQ genetic risk for CD, whereas, for those with the highest genetic risk, higher Staphylococcus spp. and Bacteroides fragilis were identified. However, the method of infant-feeding influenced the composition of the microbiota, with breast milk favoring Clostridium leptum, Bifidobacterium longum and Bifidobacterium breve gut colonization, therefore slightly switching the fecal microbiome toward the one identified in infants with low HLA-DQ genetic risk [80].

3. Bifidobacteria and Lactobacilli Strains: Few “Paladins” in the Pathogenetic Joust?

In the pursuit of the best microbial candidate for disease immunomodulation, a few Bifidobacteria strains have been studied with considerable results. For example, in an in vitro model using peripheral blood mononuclear cell (PBMCs), both Bifidobacterium longum ES1 and Bifidobacterium bifidum ES2 have been shown to downregulate the Th1 pathway typical of CD [81].

In addition, Lindfors et al. assessed whether Bifidobacterium lactis is capable of neutralizing the toxicity of gliadin. In Caco-2 cells, they found that this strain was at least able to reduce the epithelial permeability triggered by gluten [82].
Laparra et al. evaluated *Bifidobacterium longum* CECT 7347 in a murine model of CD, and they found that this specific strain not only diminishes pro-inflammatory cytokine synthesis, such as tumor necrosis factor-alfa (TNF-alfa), but it also reduces jejunal architecture damage [83]. Another group has demonstrated that *Bifidobacterium longum* strain NCC2705 produces a serine protease inhibitor with immune-modulating features, i.e., attenuating gliadin-induced histological damage in NOD/DQ8 mice [84].

An alternative *Bifidobacterium*, *B. infantis*, seems to decrease Paneth cells and expression of alpha-defensin-5 on electronic microscopy of duodenal biopsy when administered in active CD [85]. Paneth cells are key masters of gut homeostasis in innate immunity against noxious pathogens through the release of defensins, lysozyme and phospholipase [86]. Furthermore, some evidence concerning the protective effect of *Lactobacillus casei* DN-114001 and *E. coli strain Nissen 1917* on gut barrier function has been reported [87].

D’Arienzo et al. analyzed the effect of *Lactobacillus casei*, *Lactobacillus paracasei* and *Lactobacillus fermentum* in a transgenic mouse model expressing human DQ8. They found that *L casei* reduces TNF-alfa secretion and related villous blunting, while both *L paracasei* and *L fermentum* determine increased antigen-specific TNF-alfa. This suggests that, depending on the strain and on the experimental model, probiotics may have either proinflammatory or immunomodulatory properties [88,89].

### 4. Focus on Other Species and Strains: The Joust Gets Hectic

Several other bacterial species and specific strains have been studied in regard to their possible link with CD pathogenesis. As *Bacteroides fragilis* clones expressing metalloproteases were often reported in patients with CD, this might underscore an anticipated role played in CD pathogenesis. *B. fragilis* strains carrying metalloprotease genes may lead to increased intestinal permeability and production of gliadin immunogenic peptides. Furthermore, these peptides could maintain or even intensify their ability to provoke an inflammatory response mediated by TNF-alfa. These increases in TNF-alfa production by epithelial cells could have deleterious effects that fuel both innate and adaptive immunity in CD onset [90].

Some *Prevotella* species, *Lachnoanaerobaculum umeaense* and *Actinomyces Graevenitzii*, were isolated from CD jejunal biopsies. These species could trigger an IL-17A-driven immune response [91]. This emphasizes the possibility that the increased IL-17A response seen in active CD could be in part attributable to host-microbiota interactions, and this might also explain why the IL-17A mucosal response in CD is not consistent in some CD patients [92].

*Neisseria flavescens* determines inflammation and induces disturbances in the mitochondrial chain processes of Caco-2 epithelial cells. This latter metabolic alteration seems to be partly corrected when *Lactobacillus paracasei* CBA is administered [93]. Another study involving *N. flavescens* showed that five different strains isolated from adults with untreated CD led to an inflammatory activation of both human and murine dendritic cells (DC) [94]. Nevertheless, it is not clear whether *N. flavescens* causes inflammation, or the inflammatory process occurring in the gut of CD patients may favor its colonization, which then simply maintains an activated proinflammatory response.

In addition, it has been demonstrated by Galipeau et al. that gut microbiota can either reduce or exacerbate gliadin-induced damage in a mouse model of CD [95]. In this study, the expansion of the Proteobacteria phylum caused more severe intestinal damage induced by gluten. This could possibly be explained by the fact that the intestinal mucus layer is more penetrable to bacteria and toxins where Proteobacteria prevail [96]. Similar evidence comes from a study on Caco-2 cells from Spain. *Enterobacteriaceae* (belonging to the Proteobacteria phylum) were found to act similarly to gliadins regarding DC maturation, i.e., attachment, spreading and pro-inflammatory cytokine polarization. On the other hand, *Bifidobacterium longum* CECT 7347 counterbalanced IFN-production as a consequence of gliadin stimulation and increased IL-10 release [97]. Altogether, these evidences underline the importance of the biological milieu of the intestinal lumen for disease advancement.
5. Microbiome-Derived Gluten-Degrading Enzymes: Opportunity for Prevention and Alternative Treatment

Another issue to consider is the capacity of the enzyme machinery belonging to the gut microbiome to completely digest gluten. To this extent, it is of note that, after the bacterial proteolytic degradation of gliadin, peptides could still be toxic and eventually cross the intestinal barrier more easily [98]. However, few in vitro studies have revealed that microbiota components, specifically *Bifidobacteria*, can degrade proinflammatory gluten peptides in the small intestine, thus reducing their immunogenic potential [82,99]. In one recent study, some *Lactobacilli* were able to digest in vitro amylase-trypsin inhibitors (ATIs), non-gluten wheat proteins that induce an innate immune response through the Toll-like receptor 4 (TLR4)–MD2–CD14 mechanisms. It is of note that the administration of *Lactobacilli* species (*Lactobacillus salivarius* H32.1, *Lactobacillus mucosae* D5a1 and *Lactobacillus rhamnosus* LE3) decreased both inflammation and permeability stimulated by ATIs [100].

Along with the bacterial component of the gut microbiome, enzymes able to digest gluten can also be elaborated by some eukaryotes. Papista et al. studied the influence of *Saccharomyces boulardii* KK1 supplementation in an animal model of gluten enteropathy (BALB/c mice). This intervention allowed the hydrolyzation of toxic gliadin peptides and counterbalanced both enteropathy and pro-inflammatory cytokine production [101]. In line with these data, another group has shown degrading activities toward toxic gluten epitopes by oral commensal bacteria such as *Rothia* spp, *Actinomyces odontolyticus*, *Neisseria mucosa* and *Capnocytophaga sputigena* [102]. Currently, some drugs based on degrading enzymes from bacteria and fungi have been used in clinical trials with diverse results [103].

It is well established that compliance to the GFD is difficult [104], and, for this reason, there is a great expectation among CD patients for drug-based therapies [105]. In light of these challenges, these findings on gluten-degrading activities by specific microbial strains might pave the way for a probiotics-based complementary therapy of CD in the years to come.

6. Cross-Sectional Studies on CD and the Microbiome

Studies of electron microscopy scans of the small intestine have shown that some bacteria were most frequently detectable in young patients with CD during the so-called Swedish epidemics [106,107]. These rod-shaped bacteria adhering to the epithelial lining in the small intestine were commonly seen in children with CD but not in controls.

In 2007, a Spanish study showed that the diversity of stool microbiota was significantly higher in CD children than in healthy controls, and that *Bifidobacteria* showed a significantly higher species diversity in healthy children than in CD [108]. In the same year, the same group found (using small bowel biopsy samples) that the proportions of total bacteria and Gram-negative bacteria were significantly higher in CD children with active disease than in controls. *Lactobacillus–Bifidobacterium* were significantly reduced while *Bacteroides–E. coli* were significantly increased in active CD compared with controls [109]. Collado et al. showed that *Clostridium leptum*, *Bacteroides*, *Staphylococcus* and *E. coli* were significantly more abundant in stool and biopsy samples of pediatric CD patients than in healthy controls. Conversely, *Bifidobacterium* appeared significantly lower in feces of CD children as well as in biopsies compared to control children [110].

Another study on fecal samples also showed that *Bacteroides* and *Prevotella* were higher in untreated pediatric CD than in controls, whereas *Clostridium histolyticum*, *Clostridium lituseburenses* and *Faecalibacterium prausnitzii* were higher in healthy individuals than in CD children. According to previous data, *Bifidobacteria* as well were significantly reduced in untreated CD [111]. Active CD patients were reported to have a higher abundance of *Bacteroides fragilis* and a lower abundance of *Bacteroides ovatus* than controls [90].

In 2010, a study from Italy pinpointed that a higher diversity in dominant microbiota characterized CD children compared to controls. In addition, *Bacteroides* were significantly higher in CD compared to controls [112]. A subsequent study from Spain demonstrated that *Proteobacteria* were more abundant
in duodenal biopsies of active CD children than in those of controls [113]. Nevertheless, two different studies from Finland, one study from the Netherlands and one more study from Spain did not replicate previous findings, revealing no significant differences in small bowel biopsies concerning the amounts of frequencies of bacteria identified between the CD subjects and controls [71,114–116].

In adult subjects, researchers from Spain found that bacterial richness in the upper small intestinal mucosa is higher in adults than in children with CD [117]. However, in another work published in the same year, the authors demonstrated that *Bifidobacterium bifidum* was significantly higher in the stool samples of untreated CD patients than in those of healthy adults [118]. In another study, *Helicobacter* and *Megapassaera genera* were highly abundant in duodenal biopsy samples from adult CD patients compared to both first-degree relatives and the control group. Conversely, *Barnesiella* were higher in controls compared to CD and first-degree relatives in duodenal samples, while *Dorea, Akkermansia* and *Prevotella* genera were higher in fecal samples from controls compared to CD [119]. In 2016, D’Argenio et al. demonstrated that *Proteobacteria* were more abundant in samples from duodenal biopsies of adult subjects, while *Firmicutes* and *Actinobacteria* were not as well represented in active CD compared to controls. In the *Neisseriales* order, the *Neisseriaceae* family and the *Neisseria* genus were significantly more present in active CD patients than in controls [94].

However, a word of caution concerning the studies mentioned above must be noted. Studies focused on duodenal and/or jejunal microbiome in CD are scarce and often present contrasting results. Additionally, differences in mucosal microbiome composition may represent the consequence of an inflamed CD mucosa, rather than a contribution to CD pathogenesis. Ideally, comparable intestinal and fecal microbiome analyses in the same subject may shed light on possible differences to be considered in interpreting these data.

Adult patients with *dermatitis herpetiformis* seem to share more similar microbiota with control subjects than those with other clinical features of CD. In fact, patients with gastrointestinal symptoms had a higher amount of *Proteobacteria* than patients with another manifestation of the disease (i.e., *dermatitis herpetiformis*) and the control subjects [120]. This finding allows speculation into the possibility that microbiota might drive the symptoms of CD, which might also explain its protean clinical features.

In regard to specific pathogenic features of bacterial strains associated with CD, studies from Spain highlighted that virulence-gene carriage was higher in *E. coli*, in samples isolated from the stool of children with CD when compared to healthy controls [121], and that the methicillin-resistant gene (mecA) was most frequently identified in *Staphylococcus epidermidis* isolated from stools of active CD than in those from controls [122]. All cross-sectional studies are recapitulated in Table 1.

To summarize, considering all these cross-sectional studies in a comprehensive fashion, it should be pointed out that the highly individual-specific microbial profiles may greatly impact the interpretation of the results, especially when evaluated on relatively small groups (a common feature of many studies published to date). Moreover, another limitation of these results is the spurious healthy control group, especially in regard to patients who underwent an upper GI endoscopy because of signs or symptoms of disease, the microbiomes of these individuals might have hosted unpredictable microbiota alterations. In addition, a strong limitation does apply for those studies that utilized PCR-D/TGGE analysis (see Table 1), solely detecting the most-represented bacteria and therefore underestimating microbiota diversity. Finally, and specifically for the mucosa-associated microbiome, it is unclear whether changes in microbiota strains colonizing the duodenal mucosa influenced by environmental risk factors (i.e., infant-feeding practice, exposure to antibiotics and use of anti-acids, etc.) could be the cause of CD, or that structural and histological changes characterizing the celiac enteropathy are responsible for secondary shifts in the composition of the adherent microbiota. Despite all these limitations, one might conclude that a decrease in *Bifidobacteria* and an increase in *Bacteroides* seem to be a somewhat common denominator of a few studies, both on feces and on mucosal biopsies.
Table 1. Cross-sectional studies on Microbiota and subjects with celiac disease (CD).

| Author and Reference | Journal                  | Year   | Population | Country | Samples                  | Methods                                      | Significant Findings                                                                                                                                                                                                 |
|----------------------|--------------------------|--------|------------|---------|--------------------------|----------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Sanz et al. [108]    | FEMS Immunol Med Microbiol | 2007   | Children   | Spain   | Fecal samples             | DGGE                                         | The diversity of stool microbiota was significantly higher in celiac children than in healthy controls. The *Bifidobacterium* population showed a significantly higher species diversity in healthy children than in celiac patients.     |
| Nadal et al. [109]   | Journal of Medical Microbiology | 2007   | Children   | Spain   | Duodenal biopsy          | Molbiol FISH                                 | The proportions of total bacteria and Gram-negative bacteria were significantly higher in CD patients with active disease than in controls. The ratio of *Lactobacillus*-*Bifidobacterium* to *Bacteroides*-*E. Coli* was significantly reduced in celiac patients with active disease compared with controls. |
| Sánchez et al. [121] | BMC Gastroenterology     | 2008   | Children   | Spain   | Fecal samples            | VRBD agar, API20E system, PCR                | Virulence-gene carriage was higher in *E. coli* isolated from CD patients compared to healthy controls.                                                                                                               |
| Collado et al. [110] | J Clin Pathol           | 2010   | Children   | Spain   | Fecal samples and duodenal biopsy | qPCR                                         | *Clostridium leptum* and *Bacteroides* were significantly more abundant in feces and biopsies of CD patients than in healthy controls. *E. coli* and *Staphylococcus* were significantly higher in stool and biopsy samples of CD patients compared to controls, *Bifidobacterium* were significantly lower in feces of CD patients as well as in biopsies compared to control children. |
| Ou et al. [107]      | Am J Gastroenterol       | 2009   | Children   | Spain   | Duodenal biopsy          | 16s RNA amplification                        | *Bacteroides* and *Prevotella* were higher in untreated CD patients than in controls. *Clostridium histolyticum*, *Clostridium lituseburense* and *Fecalibacterium prausnitzii* were higher in healthy individuals than in CD patients. *Bifidobacteria* were significantly reduced in untreated CD patients. |
| De Palma et al. [111] | BMC Microbiology       | 2010   | Children   | Spain   | Fecal samples            | Molbiol FISH                                 | Small intestine microbiota from CD patients did not differ from controls.                                                                                                                                              |
| Schippa et al. [112] | BMC Microbiology       | 2010   | Children   | Italy    | Duodenal biopsy          | TGGE                                         | *Bacteroides* and *Prevotella* were higher in untreated CD patients than in controls. *Clostridium histolyticum*, *Clostridium lituseburense* and *Fecalibacterium prausnitzii* were higher in healthy individuals than in CD patients. *Bifidobacteria* were significantly reduced in untreated CD patients. |
| Nistal et al. [118]  | Biochimie               | 2012   | Adults     | Spain   | Fecal samples            | DGGE                                         | A higher diversity in dominant microbiota was found in CD patients compared to controls. *Bacteroides* were significantly higher in CD compared to controls.                                                                            |
| Sánchez et al. [122] | J Clin Pathol           | 2012   | Children   | Spain   | Fecal samples            | PCR ABI PRISM-310X Gene Analyzer            | *Staphylococcus epidermidis* and *haemolyticus* were more represented in the microbiota of active CD. *Staphylococcus spp.* diversity was higher in active CD patients than in controls. The methicillin-resistant gene (*mecA*) was most frequently identified in *S. epidermidis* isolates from active CD than in those from controls. |
| Sánchez et al. [90]  | Applied and Environmental Microbiology | 2012   | Children   | Spain   | Fecal samples            | Schaedlr agar, 16S rRNA amplification        | Active CD patients had a higher abundance of *Bacteroides fragilis* and a lower abundance of *Bacteroides ovatus* than controls.                                                                                           |
Table 1. Cont.

| Author and Reference | Journal | Year | Population | Country | Samples | Methods | Significant Findings |
|----------------------|---------|------|------------|---------|---------|---------|----------------------|
| Nistal et al. [117]  | Inflamm Bowel Dis 2012 | Children (<i>n = 13</i> and Adults <i>n = 15</i>) | Spain | Duodenal biopsy | 16S rRNA amplification | Bacterial richness in the upper small intestinal mucosa was higher in adults than in children. |
| Kalliomaki et al. [71] | JPGN 2012 | Children <i>(n = 19)</i> | Finland | Duodenal biopsy | qPCR | No significant differences in the amounts or frequencies of bacteria were identified between the study groups. |
| Sánchez et al. [113] | Applied and Environmental Microbiology 2013 | Children <i>(n = 40)</i> | Spain | Duodenal biopsy | 16S rRNA amplification | Increased diversity of the cultivable mucosa-associated bacteria from CD patients compared to the diversity of bacteria from the controls. <i>Proteobacteria</i> were more abundant in active CD patients than in controls. |
| Cheng et al. [114] | BMC Gastroenterology 2013 | Children <i>(n = 20)</i> | Finland | Duodenal biopsy | HITChip | None of the 65 genus-like bacteria was found to be significantly more or less abundant between CD versus healthy controls. |
| Wacklin et al. [120] | Inflamm Bowel Dis 2013 | Adult <i>(n = 51)</i> | Finland | Duodenal biopsy | PCR- DGGE | Patients with CD presenting <i>Dermatitis Herpetiformis</i> shared more similar microbiota with controls than those with other clinical features of CD. Patients with GI symptoms had a higher amount of <i>Proteobacteria</i> than the patients with another manifestation of the disease or the control subjects. |
| de Meij et al. [115] | Scandinavian Journal of Gastroenterology 2013 | Children <i>(n = 42)</i> | Netherlands | Duodenal biopsy | 16S-23S ISPRO PCR | No relevant differences in small bowel mucosal microbiome composition and diversity index was found between children with untreated CD and control. |
| Nistal et al. [116] | Journal of Applied Microbiology 2016 | Adults <i>(n = 18)</i> | Spain | Duodenal biopsy | 16S rRNA gene pyrosequencing | No differences in the duodenal microbiota between untreated CD patients and non-CD controls. |
| D’Argenio et al. [94] | Am J Gastroenterol 2016 | Adult <i>(n = 35)</i> | Italy | Duodenal biopsy | 16s next generation sequencing | <i>Proteobacteria</i> were more abundant and <i>Firmicutes</i> and <i>Actinobacteria</i> less abundant in active CD than in controls. <i>Neisseriales</i> order, the <i>Neisseriaceae</i> family, and the <i>Neisseria</i> genus were significantly more abundant in active CD patients than in controls. |
| Bodkhe et al. [119] | Front. Microbiol 2019 | Adults <i>(n = 47)</i> | India | Duodenal biopsy and fecal samples | Illumina MiSeq sequencing | In the CD group, <i>Helicobacter</i> and <i>Megasphaera</i> genera were highly abundant compared to both first-degree relatives and control group in duodenal samples. <i>Barnesiella</i> was higher in controls compared to CD and first-degree relatives in duodenal samples. <i>Dorea, Akkermansia</i> and <i>Prevotella</i> genera were higher in fecal samples from controls compared to CD. |

DDGE: Denaturing Gradient Gel Electrophoresis; FISH: Fluorescent in situ hybridization; VRBD: Violet Red Bile Dextrose; API: Analytical profile index; qPCR: quantitative polymerase chain reaction; TGGE: Temperature gradient gel electrophoresis; HITChip: Human Intestinal Tract Chip.
7. Prospective Cohort Studies on Microbiome in CD

The only chance to detect a contributory microbial signature of CD and to mechanistically correlate potential environmental factors involved in disease onset is to follow-up cohorts of infants at risk for CD in a prospective manner. It has been previously reported that HLA genotype per se affects the gut microbiota of infants at family risk for CD, with DQ2-positive subjects displaying higher abundance of Firmicutes and Proteobacteria and lower abundance of Actinobacteria [123]. Infants with high genetic risk for CD showed a higher prevalence over time of Bacteroides vulgatus, while those with low genetic risk displayed a higher prevalence of Bacteroides ovatus, Bacteroides plebeius and Bacteroides uniformis [124].

Sellitto et al. analyzed stool samples from a relatively small number of subjects at several time-points (7 days and 30 days, 6 months, 8 months, 10 months, 12 months, 18 months and 24 months). Their data suggested significant differences between the developing microbiota of infants with a genetic predisposition for CD compared to those from infants with a non-selected genetic background. In their proof-of-concept study, they recruited infants with a genetic predisposition for CD and assessed them prospectively until 24 months of age. 16s gene analysis proved that compared with the low-risk subjects, infants carrying the CD-associated HLA had increased Firmicutes and Proteobacteria, while Actinobacteria and Bacteroidetes were significantly restricted. Additionally, they also found that stool microbiota in these DQ2+/DQ8+ children did not stabilize, nor was it similar to adult microbiota at one year of age, and this feature remained at 24 months of age. In the only infant who developed CD during the follow-up, Sellitto et al. found a lactate peak between 6 and 12 months of age, speculating about a possible microbiome disturbance at that time [125].

Two more studies have been published from the PROFICEL prospective cohort in Spain by the same group. When researchers examined stool samples from infants at genetic risk for CD at 4 and 6 months of age, those who did not develop CD showed an increased bacterial diversity over time. Furthermore, a higher abundance of Bifidobacterium longum was found in control children, while higher levels of Bifidobacterium breve and Enterococcus spp. were found in those who developed CD [68]. When researchers examined stool samples from infants at genetic risk within the first week of life, and at 4 months and at 6 months of age, enterotoxigenic E. coli (ETEC) were found more often in infants with the highest genetic risk versus those with a low or intermediate risk among breastfed infants. Among infants on formula feeding, on the other hand, a higher number of ETEC was also identified in infants with a high genetic risk versus those of intermediate risk [79]. Albeit limited to fewer time-point assessments (4 months and 6 months), another prospective study from Finland did not find any significant difference in fecal microbiota composition between children who later developed CD and control children without disease or associated autoantibodies [126]. All prospective studies are summarized in Table 2.
Table 2. Prospective studies on microbiota development in subjects at risk for CD.

| Author         | Journal                                      | Year | Population | Country | Samples      | Methods            | Age of Sampling | Significant Findings                                                                 |
|----------------|----------------------------------------------|------|------------|---------|--------------|--------------------|-----------------|--------------------------------------------------------------------------------------|
| Sanchez et al. | Applied and environmental microbiology       | 2011 | Children   | Spain   | Fecal samples| DGGE               | 7 days, 1 month and 4 months | The Bacteroides diversity index was higher in formula-fed infants than in breast-fed infants, infants with high genetic risk showed a higher prevalence of *B. vulgatus*, while those with low genetic risk displayed a higher prevalence of *B. ovatus*, *B. plebeius*, and *B. uniformis*. |
| Sellitto et al. | PLoS One                                     | 2012 | Children   | USA     | Fecal samples| Roche/454 FLX pyrosequencing | 7 and 30 days, 6 months, 8 months, 10 months, 12 months, 18 months and 24 months | Genetically at-risk for CD enrolled in this study were characterized by a low abundance of members of the phylum Bacteroidetes, one infant who developed CD showed high levels of lactate between 6 and 12 months of age. |
| Olivares et al. | Microbiome                                   | 2018 | Children   | Spain   | Fecal samples| Illumina MiSeq sequencing | 4 months and 6 months | Children not developing CD showed rising bacterial diversity over time. A higher abundance of *Bifidobacterium longum* was found in control children while higher *Bifidobacterium breve* and *Enterococcus* spp. were found in those who developed CD. |
| Olivares et al. | Gut Microbes                                 | 2018 | Children   | Spain   | Fecal samples| 16S rRNA amplification     | 7 days, 1 month and 4 months | A higher prevalence of enterotoxigenic E. coli (ETEC) was found in infants with the highest genetic risk compared either to those with a low or intermediate risk |
| Rintala et al. | Scandinavian Journal of Gastroenterology     | 2018 | Children   | Finland | Fecal samples| Illumina MiSeq sequencing | 9 months and 12 months | No statistically significant differences in microbiota were found between children who later developed CD and the control children without disease or associated autoantibodies. |
8. Conclusions

Current evidence into the composition of the intestinal microbiome and its role as a causative trigger for CD is highly heterogeneous and contradictory. This is most frequently due to the limited number of cross-sectional and prospective studies performed, small sample sizes and different methodologies applied (fluorescence in situ hybridization-PCR, denaturing gradient gel electrophoresis and 16 s ribosomal RNA sequencing). In the recent past, molecular microbiology has developed based on the analysis of bacterial DNA, therefore bypassing the shortfall of microbiome analysis limited to its cultivable component. In-depth examination of the gut microbiome and identification of all strains is now mainly obtained with sequencing-based techniques [127].

However, a mechanistic analysis on how specific bacterial strains may influence intestinal health has not succeeded in considering the system biology network that plays a pivotal role in disease development. The future scenarios of biomedical science will take advantage of recognizing the unique routes that lead from health to disease, according to what has been called “precision medicine”. In the pursuit of precision medicine applied to CD, a multicenter, prospective longitudinal study called CDGEMM (Celiac Disease, Genomic, Environmental, Microbiome and Metabolomic Study) is ongoing in the USA, Italy and Spain [128,129]. CDGEMM uses a multi-omic analysis approach to identify early changes in the gut microbiome of infants genetically predisposed to CD and to monitor metatranscriptomic profiles over time, correlating those profiles to other environmental factors such as mode of delivery, feeding patterns and antibiotic exposure. This study will also deeply characterize the “metabotypes” (microbe-derived metabolomes) of these “at-risk infants” to be integrated with multi-omics profiles in the framework of system biology.

In conclusion, even though studies in both pediatric and adult patients with CD have suggested an association between altered microbiota and CD, a specific microbial signature has not been recognized. Multi-omics data from ongoing longitudinal cohort studies are eagerly awaited to further clarify this decisive field for precision medicine and primary prevention in CD.

Author Contributions: F.V. performed the literature search and drafted the paper. S.C. and A.F. carefully reviewed the draft and edited the paper.

Funding: This research received no external funding.

Conflicts of Interest: All the authors have no conflicts of interest relevant to this article.

References

1. Dicke, W.K.; Weijers, H.A.; van de Kamer, J.H. Coeliac disease. The presence in wheat of a factor having a deleterious effect in cases of coeliac disease. Acta Paediatr. Stockh. 1953, 42, 34–42. [CrossRef]
2. Lionetti, E.; Castellaneta, S.; Francavilla, R.; Pulvirenti, A.; Tonutti, E.; Amarri, S.; Barbato, M.; Barbera, C.; Barera, G.; Bellantoni, A.; et al. Introduction of gluten, HLA status, and the risk of celiac disease in children. N. Engl. J. Med. 2014, 371, 1295–1303. [CrossRef] [PubMed]
3. Singh, P.; Arora, A.; Strand, T.A.; Leffler, D.A.; Catassi, C.; Green, P.H.; Kelly, C.P.; Ahuja, V.; Makharia, G.K. Global Prevalence of Celiac Disease: Systematic Review and Meta-analysis. Clin. Gastroenterol. Hepatol. 2018, 16, 823–836. [CrossRef] [PubMed]
4. Cataldo, F.; Pitaresi, N.; Accomando, S.; Greco, L. Epidemiological and clinical features in immigrant children with coeliac disease: An Italian multicentre study. Dig. Liver. Dis. 2004, 36, 722–729. [CrossRef] [PubMed]
5. Fasano, A. Celiac disease: How to handle a clinical chameleon. N. Engl. J. Med. 2003, 348, 2568–2570. [CrossRef] [PubMed]
6. Catassi, C.; Kryszak, D.; Bhatti, B.; Sturgeon, C.; Helzlouer, K.; Clipp, S.L.; Gelfond, D.; Puppa, E.; Sferruzza, A.; Fasano, A. Natural history of celiac disease autoimmunity in a USA cohort followed since 1974. Ann. Med. 2010, 42, 530–538. [CrossRef]
7. Trovato, C.M.; Montuori, M.; Anania, C.; Barbato, M.; Vestri, A.R.; Guida, S.; Oliva, S.; Mainiero, F.; Cucchiara, S.; Valitutti, F. Are ESPGHAN “biopsy-sparing” guidelines for celiac disease also suitable for asymptomatic patients? *Am. J. Gastroenterol.* **2015**, *110*, 1485–1489. [CrossRef]

8. Tiberti, C.; Montuori, M.; Panimolle, F.; Trovato, C.M.; Anania, C.; Valitutti, F.; Vestri, A.R.; Lenzi, A.; Cucchiara, S.; Morano, S. Screening for Type 1 Diabetes-, Thyroid-, Gastric-, and Adrenal-Specific Humoral Autoimmunity in 529 Children and Adolescents with Celiac Disease at Diagnosis Identifies as Positive One in Every Nine Patients. *Diabetes Care* **2017**, *40*, e10–e11. [CrossRef]

9. Catassi, C.; Gatti, S.; Lionetti, E. World perspective and celiac disease epidemiology. *Dig. Dis.* **2015**, *33*, 141–146. [CrossRef]

10. Ivarsson, A.; Myléus, A.; Norström, F.; Van der Pals, M.; Rosén, A.; Högberg, L.; Danielsson, L.; Halvarsson, B.; Hammarroth, S.; Hernell, O.; et al. Prevalence of childhood celiac disease and changes in infant feeding. *Pediatrics* **2013**, *131*, e687–e694. [CrossRef]

11. Vriezinga, S.L.; Auricchio, R.; Bravi, E.; Castillejo, G.; Chmielewska, A.; Crespo Escobar, P.; Kolaček, S.; Koletzko, S.; Korponay-Szabó, I.R.; Mummert, E.; et al. Randomized feeding intervention in infants at high risk for celiac disease. *N. Engl. J. Med.* **2014**, *371*, 1304–1315. [CrossRef] [PubMed]

12. Greco, L.; Romino, R.; Coto, I.; Di Cosmo, N.; Percopo, S.; Maglio, M.; Paparo, F.; Gasperi, V.; Limongelli, M.G.; Cotichini, R.; et al. The first large population based twin study of coeliac disease. *Gut* **2002**, *50*, 624–628. [CrossRef] [PubMed]

13. Valitutti, F.; Fasano, A. Breaking Down Barriers: How Understanding Celiac Disease Pathogenesis Informed the Development of Novel Treatments. *Dig. Dis. Sci.* **2019**, *64*, 1748–1758. [CrossRef] [PubMed]

14. Maruvada, P.; Leone, V.; Kaplan, L.M.; Chang, E.B. The Human Microbiome and Obesity: Moving beyond Associations. *Cell Host Microbe* **2017**, *22*, 589–599. [CrossRef]

15. Knip, M.; Honkanen, J. Modulation of Type 1 Diabetes Risk by the Intestinal Microbiome. *Curr. Diab. Rep.* **2017**, *17*, 105. [CrossRef]

16. Ni, J.; Wu, G.D.; Albenberg, L.; Tomov, V.T. Gut microbiota and IBD: Causation or correlation? *Nat. Rev. Gastroenterol. Hepatol.* **2017**, *14*, 573–584. [CrossRef]

17. Kareva, I. Concise Review: Metabolism and Gut Microbiota in Cancer Immunoediting, CD8 Treg Ratios, Immune Cell Homeostasis, and Cancer (Immuno)Therapy. *Stem Cells* **2019**. [CrossRef]

18. Costa, M.; Weese, J.S. Methods and basic concepts for microbiota assessment. *Vet. J.* **2019**, *249*, 10–15. [CrossRef]

19. Amrane, S.; Raout, D.; Lagier, J.C. Metagenomics, culturoomics, and the human gut microbiota. *Expert Rev. Anti-Infect. Ther.* **2018**, *16*, 373–375. [CrossRef]

20. Holleran, G.; Lopotuso, L.R.; Ianiero, G.; Pecere, S.; Pizzoferrato, M.; Petito, V.; Graziani, C.; McNamara, D.; Gasbarrini, A.; Scaldaferrà, F. Gut microbiota and inflammatory bowel disease: So far so gut! *Minerva Gastroenterol. Dietol.* **2017**, *63*, 373–384.

21. Gilbert, J.A.; Blaser, M.J.; Caporaso, J.G.; Jansson, J.K.; Lynch, S.V.; Knight, R. Current understanding of the human microbiome. *Nat. Med.* **2014**, *20*, 392–400. [CrossRef] [PubMed]

22. Duffy, L.C.; Raiten, D.J.; Hubbard, VS.; Starke-Reed, P. Progress and challenges in developing metabolic footprints from diet in human gut microbial metabolism. *J. Nutr.* **2015**, *145*, 1123S–1130S. [CrossRef] [PubMed]

23. Gibiino, G.; Ianiero, G.; Cammarota, G.; Gasbarrini, A. The gut microbiota: Its anatomy and physiology over a lifetime. *Minerva Gastroenterol. Dietol.* **2017**, *63*, 329–336. [PubMed]

24. Bibbò, S.; Ianiero, G.; Giorgio, V.; Scaldaferrà, F.; Masucci, L.; Gasbarrini, A.; Cammarota, G. The role of diet on gut microbiota composition. *Eur. Rev. Med. Pharmacol. Sci.* **2016**, *20*, 4742–4749.

25. Ducarmon, Q.R.; Zwittink, R.D.; Hornung, B.V.H.; van Schaik, W.; Young, V.B.; Kuijper, E.J. Gut Microbiota and Colonization Resistance against Bacterial Enteric Infection. *Microbiol. Mol. Biol. Rev.* **2019**, *83*, e00007-19. [CrossRef]

26. Odenwald, M.A.; Turner, J.R. The intestinal epithelial barrier: A therapeutic target? *Nat. Rev. Gastroenterol. Hepatol.* **2017**, *14*, 9–21. [CrossRef]

27. Woo, V.; Alenghat, T. Host-microbiota interactions: Epigenomic regulation. *Curr. Opin. Immunol.* **2017**, *44*, 52–60. [CrossRef]

28. Macpherson, A.J.; Harris, N.L. Interactions between commensal intestinal bacteria and the immune system. *Nat. Rev. Immunol.* **2004**, *4*, 478–485. [CrossRef]
29. Rinninella, E.; Raoul, P.; Cintoni, M.; Franceschi, F.; Miggiano, G.A.D.; Gasbarrini, A.; Mele, M.C. What is the Healthy Gut Microbiota Composition? A Changing Ecosystem across Age, Environment, Diet, and Diseases. *Microorganisms* 2019, 7, 14. [CrossRef]

30. Yatsunenko, T.; Rey, F.E.; Manary, M.J.; Trehan, I.; Dominguez-Bello, M.G.; Contreras, M.; Magris, M.; Hidalgo, G.; Baldassano, R.N.; Anokhin, A.P.; et al. Human gut microbiome viewed across age and geography. *Nature* 2012, 486, 222–227. [CrossRef]

31. Aagaard, K.; Ma, J.; Antony, K.M.; Ganu, R.; Petrosino, J.; Versalovic, J. The placenta harbors a unique microbiome. *Sci. Transl. Med.* 2014, 6, 237ra65. [CrossRef]

32. Wilczyńska, P.; Skarżyńska, E.; Lisowska-Myjak, B. Meconium microbiome as a new source of information about long-term health and disease: Questions and answers. *J. Matern. Fetal Neonatal. Med.* 2019, 32, 681–686. [CrossRef]

33. Dominguez-Bello, M.G.; Costello, E.K.; Contreras, M.; Magris, M.; Hidalgo, G.; Fierer, N.; Knight, R. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc. Natl. Acad. Sci. USA* 2010, 107, 11971–11975. [CrossRef]

34. Azad, M.B.; Konya, T.; Maughan, H.; Guttman, D.S.; Field, C.J.; Chari, R.S.; Sears, M.R.; Becker, A.B.; Scott, J.A.; Kozyrskyj, A.L.; et al. Gut microbiota of healthy Canadian infants: Profiles by mode of delivery and infant diet at 4 months. *CMAJ* 2013, 185, 385–394. [CrossRef] [PubMed]

35. Hills, R.D., Jr.; Pontefract, B.A.; Mishcon, H.R.; Black, C.A.; Sutton, S.C.; Theberge, C.R. Gut Microbiome: Profound Implications for Diet and Disease. *Nutrients* 2019, 11, 1613. [CrossRef] [PubMed]

36. Rutayisire, E.; Huang, K.; Liu, Y.; Tao, F. The mode of delivery affects the diversity and colonization pattern of the gut microbiota during the first year of infants’ life: A systematic review. *BMC Gastroenterol.* 2016, 16, 86. [CrossRef] [PubMed]

37. Jakobsson, H.E.; Stephansson, O.; Montgomery, S.; Murray, J.A.; Ludvigsson, J.F. Pregnancy outcome and risk of celiac disease in offspring: A nationwide case-control study. *Gastroenterology* 2012, 142, 39–45. [CrossRef]

38. Emilsson, L.; Magnus, M.C.; Størdal, K. Perinatal risk factors for development of celiac disease in children, based on the prospective Norwegian Mother and Child Cohort Study. *Clin. Gastroenterol. Hepatol.* 2015, 13, 921–927. [CrossRef]

39. Mårlid, K.; Stephansson, O.; Montgomery, S.; Murray, J.A.; Ludvigsson, J.F. Pregnancy outcome and risk of celiac disease in offspring: A changing ecosystem across age, environment, diet and diseases. *Gastroenterology* 2012, 142, 39–45. [CrossRef]

40. Asakuma, S.; Hatakeyama, E.; Urashima, T.; Yoshida, E.; Katayama, T.; Yamamoto, K.; Kumagai, H.; Ashida, H.; Hirose, J.; Kitaoka, M. Physiology of consumption of human milk oligosaccharides by infant gut-associated bifidobacteria. *J. Biol. Chem.* 2011, 286, 34583–34592. [CrossRef] [PubMed]

41. Nguyen, T.T.; Kim, J.W.; Park, J.S.; Hwang, K.H.; Jang, T.S.; Kim, C.H.; Kim, D. Identification of Oligosaccharides in Human Milk Bound onto the Toxin a Carbohydrate Binding Site of Clostridium difficile. *J. Microbiol. Biotechnol.* 2016, 26, 659–665. [CrossRef] [PubMed]

42. Wang, C.; Zhang, M.; Guo, H.; Yan, J.; Liu, F.; Chen, J.; Li, Y.; Ren, F. Human Milk Oligosaccharides Protect against Necrotizing Enterocolitis by Inhibiting Intestinal Damage via Increasing the Proliferation of Crypt Cells. *Mol. Nutr. Food Res.* 2019, e1900262. [CrossRef] [PubMed]

43. Fallani, M.; Young, D.; Scott, J.; Norin, E.; Amari, S.; Adam, R.; Aguilera, M.; Khanna, S.; Gil, A.; Edwards, C.A.; et al. Intestinal microbiota of 6-week-old infants across Europe: Geographic influence beyond delivery mode, breast-feeding, and antibiotics. *J. Pediatr. Gastroenterol. Nutr.* 2010, 51, 77–84. [CrossRef] [PubMed]

44. Mårlid, K.; Ludvigsson, J.; Sanz, Y.; Ludvigsson, J.F. Antibiotic exposure in pregnancy and risk of coeliac disease in offspring: A cohort study. *BMC Gastroenterol.* 2014, 14, 75. [CrossRef]

45. Dydenschborg Sander, S.; Nybo Andersen, A.M.; Murray, J.A.; Karlstad, Ø.; Husby, S.; Størdal, K. Association Between Antibiotics in the First Year of Life and Celiac Disease. *Gastroenterology* 2019, 156, 2217–2229. [CrossRef]
47. Canova, C.; Zabeo, V.; Pitter, G.; Romor, P.; Baldovin, T.; Zanotti, R.; Simonato, L. Association of maternal education, early infections, and antibiotic use with celiac disease: A population-based birth cohort study in northeastern Italy. *Am. J. Epidemiol.* 2014, 180, 76–85. [CrossRef]

48. Mylénus, A.; Hernell, O.; Gothenors, L.; Hammarström, M.L.; Persson, L.A.; Stenlund, H.; Ivarsson, A. Early infections are associated with increased risk for celiac disease: An incident case-referent study. *BMC Pediatr.* 2012, 12, 194. [CrossRef]

49. Mårild, K.; Ye, W.; Lebwohl, B.; Green, P.H.; Blaser, M.J.; Card, T.; Ludvigsson, J.F. Antibiotic exposure and the development of coeliac disease: A nationwide case-control study. *BMC Gastroenterol.* 2013, 13, 109. [CrossRef] [PubMed]

50. Kemppainen, K.M.; Vehik, K.; Lynch, K.F.; Larsson, H.E.; Canepa, R.J.; Simell, V.; Koletzko, S.; Liu, E.; Simell, O.G.; Toppadi, J.; et al. Association Between Early-Life Antibiotic Use and the Risk of Islet or Celiac Disease Autoimmunity. *JAMA Pediatr.* 2017, 171, 1217–1225. [CrossRef]

51. Kołodziej, M.; Patro-Gołab, B.; Gieruszczak-Białek, D.; Skórzka, A.; Pieścik-Lech, M.; Baron, R.; Szajewska, H.; On behalf of the SAWANTI Working Group. Association between early life (prenatal and postnatal) antibiotic administration and coeliac disease: A systematic review. *Arch. Dis. Child.* 2019. [CrossRef]

52. Kemppainen, K.M.; Lynch, K.F.; Liu, E.; Lönnrot, M.; Simell, V.; Briese, T.; Koletzko, S.; Hagopian, W.; Rewers, M.; She, J.X.; et al. Factors that increase risk of celiac disease autoimmunity after a gastrointestinal infection in early life. *Clin. Gastroenterol. Hepatol.* 2017, 15, 694–702. [CrossRef] [PubMed]

53. Mårild, K.; Kahrs, C.R.; Tapia, G.; Størdal, K. Infections and risk of celiac disease in childhood: A prospective nationwide cohort study. *Am. J. Gastroenterol.* 2015, 110, 1475–1484. [CrossRef] [PubMed]

54. Bouziat, R.; Hinterleitner, R.; Brown, J.J.; Stencel-Baerenwald, J.E.; Ikizler, M.; Mayassi, T.; Meisel, M.; Kim, S.M.; Discepolo, V.; Prijssers, A.J. Reovirus infection triggers inflammatory responses to dietary antigens and development of celiac disease. *Science* 2017, 356, 44–50. [CrossRef]

55. Kagnoff, M.F.; Paterson, Y.J.; Kumar, P.J.; Kasarda, D.D.; Carbone, F.R.; Unsworth, D.J.; Austin, R.K. Evidence for the role of a human intestinal adenovirus in the pathogenesis of coeliac disease. *Gut* 1987, 28, 995–1001. [CrossRef]

56. Lähdeaho, M.L.; Lehtinen, M.; Rissa, H.R.; Hyöty, H.; Reunala, T.; Mäki, M. Antipeptide antibodies to adenovirus E1b protein indicate enhanced risk of celiac disease and dermatitis herpetiformis. *Int. Arch. Allergy Immunol.* 1993, 101, 272–276. [CrossRef]

57. Stene, L.C.; Honeyman, M.C.; Hoffenberg, E.J.; Haas, J.E.; Sokol, R.J.; Emery, L.; Taki, I.; Norris, J.M.; Erlich, H.A.; Eisenbarth, G.S.; et al. Rotavirus infection frequency and risk of celiac disease autoimmunity in early childhood: A longitudinal study. *Am. J. Gastroenterol.* 2006, 101, 2333–2340. [CrossRef]

58. Gatti, S.; Lionetti, E.; Balanzoni, L.; Verma, A.K.; Galeazzi, T.; Gesuata, R.; Scattolo, N.; Cinquetti, M.; Fasano, A.; Catassi, C. Increased Prevalence of Celiac Disease in School-age Children in Italy. *Clin. Gastroenterol. Hepatol.* 2019. [CrossRef]

59. Corouge, M.; Loridan, S.; Fradin, C.; Sallier, J.; Damiens, S.; Moragues, M.D.; Souplet, V.; Jouault, T.; Robert, R.; Dubucquoi, S.; et al. Humoral immunity links Candida albicans infection and celiac disease. *PLoS ONE* 2015, 10, e0121776. [CrossRef]

60. D’Argenio, V.; Casaburi, G.; Precone, V.; Pagliuca, C.; Colicchio, R.; Sarnataro, D.; Discepolo, V.; Kim, S.M.; Russo, I.; Del Vecchio Blanco, G. No Change in the Mucosal Gut Mycobioma Is Associated with Celiac Disease-Specific Microbiome Alteration in Adult Patients. *Am. J. Gastroenterol.* 2016, 111, 1659–1661. [CrossRef] [PubMed]

61. Lebwohl, B.; Nobel, Y.R.; Green, P.H.R.; Blaser, M.J.; Ludvigsson, J.F. Risk of Clostridium difficile Infection in Patients with Celiac Disease: A Population-Based Study. *Am. J. Gastroenterol.* 2017, 112, 1878–1884. [CrossRef] [PubMed]

62. Valitutti, F.; Trovato, C.M.; Montuori, M.; Cucchiara, S.C. difficile and celiac disease: The “difficile” to tell association. *Am. J. Gastroenterol.* 2018, 113, 777–778. [CrossRef] [PubMed]

63. Vaishnava, S.; Yamamoto, M.; Severson, K.M.; Ruhn, K.A.; Yu, X.; Koren, O.; Ley, R.; Wakeland, E.K.; Hooper, L.V. The antibacterial lectin RegIIIgamma promotes the spatial segregation of microbiota and host in the intestine. *Science* 2011, 334, 255–258. [CrossRef] [PubMed]
64. Martin, R.; Chamignon, C.; Mhedbi-Hajri, N.; Chain, F.; Derrien, M.; Escribano-Vázquez, U.; Garault, P.; Cotillard, A.; Pham, H.P.; Chervaux, C.; et al. The potential probiotic Lactobacillus rhamnosus CNCM I-3690 strain protects the intestinal barrier by stimulating both mucus production and cytoprotective response. Sci. Rep. 2019, 9, 5398. [CrossRef] [PubMed]
65. Van der Lugt, B.; Van Beek, A.A.; Aalvink, S.; Meijer, B.; Sovran, B.; Vermeij, W.P.; Brandt, R.M.C.; De Vos, W.M.; Savelkoul, H.F.J.; Steegenga, W.T.; et al. Akkermansia muciniphila ameliorates the age-related decline in colonic mucus thickness and attenuates immune activation in accelerated aging Erc1 Δ/Δ7 mice. Immun. Ageing 2019, 16, 6. [CrossRef] [PubMed]
66. Shen, X.; Cui, H.; Xu, X. Orally administered Lactobacillus casei exhibited several probiotic properties in artificially suckling rabbits. Asian-Australas. J. Anim. Sci. 2019. [CrossRef]
67. Fukuda, S.; Toh, H.; Hase, K.; Oshima, K.; Nakanishi, Y.; Yoshimura, K.; Tobe, T.; Clarke, J.M.; Topping, D.L.; Suzuki, T.; et al. Bifidobacteria can protect from enteropathogenic infection through production of acetate. Nature 2011, 469, 543–547. [CrossRef]
68. Peterson, D.A.; McNulty, N.P.; Guruge, J.L.; Gordon, J.I. IgA response to symbiotic bacteria as a mediator of gut homeostasis. Cell Host Microbe 2007, 2, 328–339. [CrossRef]
69. Olivares, M.; Walker, A.W.; Capilla, A.; Benítez-Páez, A.; Palau, F.; Parkhill, J.; Castillejo, G.; Sanz, Y. Gut microbiota trajectory in early life may predict development of celiac disease. Microbiome 2018, 6, 36. [CrossRef]
70. Szebeni, B.; Veres, G.; Dezsöfi, A.; Rusai, K.; Vannay, A.; Bokodi, G.; Vásárhelyi, B.; Korponay-Szabó, I.R.; Tulassay, T.; Arató, Á. Increased mucosal expression of Toll-like receptor (TLR)2 and TLR4 in coeliac disease. J. Pediatr. Gastroenterol. Nutr. 2007, 45, 187–193. [CrossRef]
71. Kalliomäki, M.; Satokari, R.; Lähteenoja, H.; Vähämiko, S.; Grönlund, J.; Routi, T.; Salminen, S. Expression of Toll-like receptors, their regulators, and their ligands in the small intestinal mucosa in celiac disease. J. Pediatr. Gastroenterol. Nutr. 2012, 54, 727–732. [CrossRef] [PubMed]
72. Otte, J.M.; Cario, E.; Podolsky, D.K. Mechanisms of cross hyporesponsiveness to Toll-like receptor bacterial ligands in intestinal epithelial cells. Gastroenterology 2004, 126, 1054–1070. [CrossRef] [PubMed]
73. Hooper, L.V.; Littman, D.R.; Macpherson, A.J. Interactions between the microbiota and the immune system. Science 2012, 336, 1268–1273. [CrossRef] [PubMed]
74. Sun, M.; Wu, W.; Liu, Z.; Cong, Y. Microbiota metabolite short chain fatty acids, GPCR, and inflammatory bowel diseases. J. Gastroenterol. 2017, 52, 1–8. [CrossRef]
75. Scholz, T.; Versteijden, C.; De Jonge, W.J. Dietary inhibitors of histone deacetylases in intestinal immunity and homeostasis. Front. Immunol. 2013, 4, 226. [CrossRef]
76. Serena, G.; Yan, S.; Camhi, S.; Patel, S.; Lima, R.S.; Sapone, A.; Leonard, M.M.; Mukherjee, R.; Nath, B.J.; Lammers, K.M.; et al. Proinflammatory cytokine interferon-γ and microbiome-derived metabolites dictate epigenetic switch between forkhead box protein 3 isoforms in coeliac disease. Clin. Exp. Immunol. 2017, 187, 490–506. [CrossRef]
77. Freire, R.; Ingano, L.; Serena, G.; Cetinbas, M.; Anselmo, A.; Sapone, A.; Sadreyev, R.I.; Fasano, A.; Senger, S. Human gut derived-organoids provide model to study gluten response and effects of microbiota-derived molecules in celiac disease. Sci. Rep. 2019, 9, 7029. [CrossRef]
78. De Palma, G.; Capilla, A.; Nadal, I.; Nova, E.; Pozo, T.; Varea, V.; Polanco, I.; Castillejo, G.; López, A.; Garrote, J.A.; et al. Interplay between human leukocyte antigen genes and the microbial colonization process of the newborn intestine. Curr. Issues Mol. Biol. 2010, 12, 1–10.
79. Olivares, M.; Benítez-Páez, A.; de Palma, G.; Capilla, A.; Nova, E.; Castillejo, G.; Varea, V.; Marcos, A.; Garrote, J.A.; Polanco, I.; et al. Increased prevalence of pathogenic bacteria in the gut microbiota of infants at risk of developing celiac disease: The PROFICEL study. Gut Microbes 2018, 9, 551–558. [CrossRef]
80. Palma, G.D.; Capilla, A.; Nova, E.; Castillejo, G.; Varea, V.; Pozo, T.; Garrote, J.A.; Polanco, I.; López, A.; Ribes-Konincx, C.; et al. Influence of milk-feeding type and genetic risk of developing coeliac disease on intestinal microbiota of infants: The PROFICEL study. PLoS ONE 2012, 7, e30791. [CrossRef]
81. Medina, M.; De Palma, G.; Ribes-Konincx, C.; Calabuig, M.; Sanz, Y. Bifidobacterium strains suppress in vitro the pro-inflammatory milieu triggered by the large intestinal microbiota of coeliac patients. J. Inflamm. 2008, 5, 19. [CrossRef] [PubMed]
82. Lindfors, K.; Blomqvist, T.; Juuti-Uusitalo, K.; Stenman, S.; Venäläinen, J.; Mäki, M.; Kaukinen, K. Live probiotic Bifidobacterium lactis bacteria inhibit the toxic effects induced by wheat gliadin in epithelial cell culture. Clin. Exp. Immunol. 2008, 152, 552–558. [CrossRef] [PubMed]

83. Laparra, J.M.; Olivares, M.; Gallina, O.; Sanz, Y. Bifidobacterium longum CECT 7347 modulates immune responses in a gliadin-induced enteropathy animal model. PLoS ONE 2012, 7, e30744. [CrossRef] [PubMed]

84. McCarville, J.L.; Dong, J.; Caminero, A.; Bermudez-Brito, M.; Jury, J.; Murray, J.A.; Duboux, S.; Steinmann, M.; Delley, M.; Tangyu, M.; et al. A Commensal Bifidobacterium longum Strain Prevents Gluten-Related Immunopathology in Mice through Expression of a Serine Protease Inhibitor. Appl. Environ. Microbiol. 2017, 83, e01323-17. [CrossRef] [PubMed]

85. Pinto-Sánchez, M.I.; Smecuol, E.C.; Temprano, M.P.; Sugai, E.; González, A.; Moreno, M.L.; Huang, X.; Bercik, P.; Cabanne, A.; Vázquez, H. Bifidobacterium infantis NLS Super Strain Reduces the Expression of α-Defensin-5, a Marker of Innate Immunity, in the Mucosa of Active Celiac Disease Patients. J. Clin. Gastroenterol. 2017, 51, 814–817. [CrossRef]

86. Gassler, N. Paneth cells in intestinal physiology and pathophysiology. World J. Gastrointest. Pathophysiol. 2017, 8, 150–160. [CrossRef]

87. Zyrek, A.A.; Cichon, C.; Helms, S.; Enders, C.; Sonnenborn, U.; Schmidt, M.A. Molecular mechanisms underlying the probiotic effects of Escherichia coli Nissle 1917 involve ZO-2 and PKCzeta redistribution resulting in tight junction and epithelial barrier repair. Cell. Microbiol. 2007, 9, 804–816. [CrossRef]

88. D’Arienzo, R.; Maurano, F.; Lavermicocca, P.; Ricca, E.; Rossi, M. Modulation of the immune response by probiotic strains in a mouse model of gluten sensitivity. Cytokine 2009, 48, 254–259. [CrossRef]

89. D’Arienzo, R.; Stefanile, R.; Maurano, F.; Mazzarella, G.; Ricca, E.; Troncone, R.; Auricchio, S.; Rossi, M. Immunomodulatory effects of Lactobacillus casei administration in a mouse model of gliadin-sensitive enteropathy. Scand. J. Immunol. 2011, 74, 335–341. [CrossRef]

90. Sánchez, E.; Laparra, J.M.; Sanz, Y. Discerning the role of Bacteroides fragilis in celiac disease pathogenesis. Appl. Environ. Microbiol. 2012, 78, 6507–6515. [CrossRef]

91. Sjöberg, V.; Sandström, O.; Hedberg, M.; Hammarström, S.; Hernell, O.; Hammarström, M.L. Intestinal T-cell responses in celiac disease—Impact of celiac disease associated bacteria. PLoS ONE 2013, 8, e53414. [CrossRef] [PubMed]

92. La Scaleia, R.; Barba, M.; Di Nardo, G.; Bonamico, M.; Oliva, S.; Nenna, R.; Valitutti, F.; Mennini, M.; Barbato, M.; Montuori, M.; et al. Size and dynamics of mucosal and peripheral IL-17A+ T-cell pools in pediatric age, and their disturbance in celiac disease. Mucosal. Immunol. 2012, 5, 513–523. [CrossRef] [PubMed]

93. Labruna, G.; Nanayakkara, M.; Pagliuca, C.; Nunziato, M.; Iaffaldano, L.; D’Argenio, V.; Colicchio, R.; Budelli, A.L.; Nigro, R.; Salvatore, P.; et al. Celiac disease-associated Neisseria flavescens decreases mitochondrial respiration in CaCo-2 epithelial cells: Impact of Lactobacillus casei CBA L74 on bacterial-induced cellular imbalance. Cell. Microbiol. 2019, 21, e13035. [CrossRef] [PubMed]

94. D’Argenio, V.; Casaburi, G.; Precone, V.; Pagliuca, C.; Colicchio, R.; Sarnataro, D.; Discepolo, V.; Kim, S.M.; Russo, I.; Del Vecchio Blanco, G.; et al. Metagenomics reveals dysbiosis and a potentially pathogenic n. flavescens strain in duodenum of adult celiac patients. Am. J. Gastroenterol. 2016, 111, 879–890. [CrossRef]

95. Galipeau, H.J.; McCarville, J.L.; Huebener, S.; Litwin, O.; Meisel, B.; Jabri, B.; Sanz, Y.; Murray, J.A.; Jordana, M.; Alaedini, A.; et al. Intestinal microbiota modulates gluten-induced immunopathology in humanized mice. Am. J. Pathol. 2015, 185, 2969–2982. [CrossRef]

96. Jakobsson, H.E.; Rodríguez-Piñeiro, A.M.; Schütte, A.; Ermund, A.; Boysen, P.; Bemark, M.; Sommer, F.; Bäckhed, F.; Hansson, G.C.; Johansson, M.E. The composition of the gut microbiota shapes the colon mucus barrier. EMBO Rep. 2015, 16, 164–177. [CrossRef]

97. De Palma, G.; Kamanova, J.; Cínova, J.; Olivares, M.; Drasarova, H.; Tuckova, L.; Sanz, Y. Modulation of phenotypic and functional maturation of dendritic cells by intestinal bacteria and gliadin: Relevance for celiac disease. J. Leukoc. Biol. 2012, 92, 1043–1054. [CrossRef]

98. Caminero, A.; Galipeau, H.J.; McCarville, J.L.; Johnston, C.W.; Bernier, S.P.; Russell, A.K.; Jury, J.; Herran, A.R.; Casqueiro, J.; Tye-Din, J.A.; et al. Duodenal bacteria from patients with celiac disease and healthy subjects distinctly affect gluten breakdown and immunogenicity. Gastroenterology 2016, 151, 670–683. [CrossRef]
109. Nadal, I.; Donat, E.; Ribes-Koninckx, C.; Calabuig, M.; Sanz, Y. Imbalance in the composition of the duodenal microbiota associated with celiac disease. J. Cell. Biochem. 2010, 109, 801–807. [CrossRef]

110. Caminero, A.; McCarville, J.L.; Zevallos, V.F.; Pigrau, M.; Yu, X.B.; Jury, J.; Galipeau, H.J.; Clarizio, A.V.; Casqueiro, J.; Murray, J.A.; et al. Lactobacilli degrade wheat amylase trypsin inhibitors to reduce intestinal dysfunction induced by immunogenic wheat proteins. Gastroenterology 2019, 156, 2266–2280. [CrossRef]

111. De Palma, G.; Nadal, I.; Medina, M.; Donat, E.; Ribes-Koninckx, C.; Calabuig, M.; Sanz, Y. Intestinal dysbiosis in celiac disease vs non-celiac disease controls. [CrossRef] [PubMed]

112. Schippa, S.; Iebba, V.; Barbato, M.; Di Nardo, G.; Totino, V.; Checchi, M.P.; Longhi, C.; Maiella, G.; Cuchiara, S.; Conte, M.P. A distinctive ‘microbial signature’ in celiac pediatric patients. BMC Microbiol. 2010, 10, 63. [CrossRef] [PubMed]

113. Sánchez, E.; Donat, E.; Ribes-Koninckx, C.; Fernández-Murga, M.L.; Sanz, Y. Duodenal-mucosal bacteria associated with celiac disease in children. Appl. Environ. Microbiol. 2013, 79, 5472–5479. [CrossRef] [PubMed]

114. Cheng, J.; Kalliomäki, M.; Heilig, H.G.; Palva, A.; Lähteenoja, H.; De Vos, W.M.; Salojärvi, J.; Sato, K.; Duodenal microbiota composition and mucosal homeostasis in pediatric celiac disease. BMC Gastroenterol. 2013, 13, 113. [CrossRef] [PubMed]

115. De Meij, T.G.; Budding, A.E.; Grasman, M.E.; Kneepkens, C.M.; Savelkoul, P.H.; Mearin, M.L. Composition and diversity of the duodenal mucosa-associated microbiome in children with untreated coeliac disease. Scand. J. Gastroenterol. 2013, 48, 530–536. [CrossRef] [PubMed]

116. Nistal, E.; Caminero, A.; Herrán, A.R.; Pérez-Andrés, J.; Vivas, S.; Ruiz de Morales, J.M.; Sáenz de Miera, L.E.; Casqueiro, J. Study of duodenal bacterial communities by 16S rRNA gene analysis in adults with active celiac disease vs non-celiac disease controls. J. Appl. Microbiol. 2016, 120, 1691–1700. [CrossRef] [PubMed]

117. Nistal, E.; Caminero, A.; Herrán, A.R.; Arias, L.; Vivas, S.; de Morales, J.M.; Calleja, S.; de Miera, L.E.; Arroyo, P.; Casqueiro, J. Differences of small intestinal bacteria populations in adults and children with/without celiac disease: Effect of age, gluten diet, and disease. Inflamm. Bowel. Dis. 2012, 18, 649–656. [CrossRef]
118. Nistal, E.; Caminero, A.; Vivas, S.; Ruiz de Morales, J.M.; Sáenz de Miera, L.E.; Rodríguez-Aparicio, L.B.; Casqueiro, J. Differences in faecal bacteria metabolism and faecal bacteria metabolism in healthy adults and celiac disease patients. *Biochimie* 2012, 94, 1724–1729. [CrossRef]

119. Bodkhe, R.; Shetty, S.A.; Dhotre, D.P.; Verma, A.K.; Bhatia, K.; Mishra, A.; Kaur, G.; Pande, P.; Bangarusanmy, D.K.; Santosh, B.P.; et al. Comparison of Small Gut and Whole Gut Microbiota of First-Degree Relatives WITH Adult Celiac Disease Patients and Controls. *Front. Microbiol.* 2019, 10, 164. [CrossRef]

120. Wacklin, P.; Kaukinen, K.; Tuovinen, E.; Collin, P.; Lindfors, K.; Partanen, J.; Mäki, M.; Mättö, J. The duodenal microbiota composition of adult celiac disease patients is associated with the clinical manifestation of the disease. *Inflamm. Bowel. Dis.* 2013, 19, 934–941. [CrossRef]

121. Sánchez, E.; Nadal, I.; Donat, E.; Ribes-Koninckx, C.; Calabuig, M.; Sanz, Y. Reduced diversity and increased virulence-gene carriage in intestinal enterobacteria of coeliac children. *BMC Gastroenterol.* 2008, 8, 50. [CrossRef] [PubMed]

122. Sánchez, E.; Ribes-Koninckx, C.; Calabuig, M.; Sanz, Y. Intestinal Staphylococcus spp. and virulent features associated with celiac disease. *J. Clin. Pathol.* 2012, 65, 830–834. [CrossRef]

123. Olivares, M.; Neef, A.; Castillejo, G.; Palma, G.D.; Varea, V.; Capilla, A.; Palau, F.; Nova, E.; Marcos, A.; Polanco, I.; et al. The HLA-DQ2 genotype selects for early intestinal microbiota composition in infants at high risk of developing celiac disease. *Gut* 2015, 64, 406–417. [CrossRef] [PubMed]

124. Sánchez, E.; De Palma, G.; Capilla, A.; Nova, E.; Pozo, T.; Castillejo, G.; Varea, V.; Marcos, A.; Garrote, J.A.; Polanco, I.; et al. Influence of environmental and genetic factors linked to celiac disease risk on infant gut colonization by Bacteroides species. *Appl. Environ. Microbiol.* 2011, 77, 5316–5323. [CrossRef] [PubMed]

125. Sellitto, M.; Bai, G.; Serena, G.; Fricke, W.F.; Sturgeon, C.; Gajer, P.; White, J.R.; Koenig, S.S.; Sakamoto, J.; Booth, D.; et al. Proof of concept of microbiome-metabolome analysis and delayed gluten exposure on celiac disease autoimmunity in genetically at-risk infants. *PLoS ONE* 2012, 7, e33387. [CrossRef] [PubMed]

126. Rintala, A.; Riikonen, I.; Toivonen, A.; Pietilä, S.; Munukka, E.; Pursiheimo, J.P.; Elo, L.L.; Arikoski, P.; Luopajärvi, K.; Schwab, U.; et al. Early fecal microbiota composition in children who later develop celiac disease and associated autoimmunity. *Scand. J. Gastroenterol.* 2018, 53, 403–409. [CrossRef]

127. Qin, J.; Li, R.; Raes, J.; Arumugam, M.; Burgdorf, K.S.; Manichanh, C.; Nielsen, T.; Pons, N.; Levenez, F.; Yamada, T.; et al. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 2010, 464, 59–65. [CrossRef]

128. Leonard, M.M.; Fasano, A. The microbiome as a possible target to prevent celiac disease. *Expert Rev. Gastroenterol. Hepatol.* 2016, 10, 555–556. [CrossRef]

129. Leonard, M.M.; Camhi, S.; Huedo-Medina, T.B.; Fasano, A. Celiac Disease Genomic, Environmental, Microbiome, and Metabolomic (CDGEMM) Study Design: Approach to the Future of Personalized Prevention of Celiac Disease. *Nutrients* 2015, 7, 9325–9336. [CrossRef]