Celiac Disease and the Microbiome

Subjects: Nutrition & Dietetics

Contributor: Francesco Valitutti

Celiac disease (CD) has most often been perceived as a pediatric condition with a peak incidence in children younger than two years of age. 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 CD.

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].

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][49][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 mycobiome 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 glycocalyx 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 mucous 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, Szebeni 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 lipotechoic 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 alfa-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.

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.

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.

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.

4. Cataldo, F.; Pitarresi, 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.

5. Fasano, A. Celiac disease: How to handle a clinical chameleon. N. Engl. J. Med. 2003, 348, 2568–2570.

6. Catassi, C.; Kryszak, D.; Bhatti, B.; Sturgeon, C.; Helzlsouer, 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.

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.

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.

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

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.

11. Vriezinga, S.L.; Auricchio, R.; Bravi, E.; Castillejo, G.; Chmielewska, A.; Crespo Escobar, P.; Kolaček, S.; Koletzko, S.; Korponay-Szabo, 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.

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.

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.

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.

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

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.

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.

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

19. Amrane, S.; Raoult, D.; Lagier, J.C. Metagenomics, culturomics, and the human gut microbiota. Expert Rev. Anti-Infect. Ther. 2018, 16, 373–375.

20. Holleran, G.; Lopetuso, L.R.; Ianiro, G.; Pecere, S.; Pizzoferrato, M.; Petito, V.; Graziani, C.; McNamara, D.; Gasbarrini, A.; Scaldaferri, 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. 2018, 24, 392–400.

22. Duffy, L.C.; Raiten, D.J.; Hubbard, V.S.; Starke-Reed, P. Progress and challenges in developing metabolic footprints from diet in human gut microbial cometabolism. J. Nutr. 2015, 145, 1123S–1130S.

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

24. Bibbò, S.; Ianiro, G.; Giorgio, V.; Scaldaferri, 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.

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

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

28. Macpherson, A.J.; Harris, N.L. Interactions between commensal intestinal bacteria and the immune system. Nat. Rev. Immunol. 2004, 4, 478–485.

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.

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.

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.

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.

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.

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.

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.

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.

37. Jakobsson, H.E.; Abrahamsson, T.R.; Jenmalm, M.C.; Harris, K.; Quince, C.; Jernberg, C.; Björkstén, B.; Engstrand, L.; Andersson, A.F. Decreased gut microbiota diversity, delayed Bacteroidetes colonisation and reduced Th1 responses in infants delivered by caesarean section. Gut 2014, 63, 559–566.

38. Decker, E.; Engelmann, G.; Findeisen, A.; Gerner, P.; Laass, M.; Ney, D.; Posovszky, C.; Hoy, L.; Hornef, M.W. Cesarean delivery is associated with celiac disease but not inflammatory bowel disease in children. Pediatrics 2010, 125, e1433–e1440.

39. Mårild, K.; 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.

40. 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.

41. 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.

42. 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.

43. 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.

44. Fallani, M.; Young, D.; Scott, J.; Norin, E.; Amarri, 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.

45. Mårild, 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.

46. Dydensborg 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.

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.

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

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.

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

51. Kołodziej, M.; Patro-Gołąb, B.; Gieruszczak-Białek, D.; Skórka, 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.

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.

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

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

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.

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.

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.

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

59. Corouge, M.; Loridant, S.; Fradin, C.; Salleron, 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.

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.

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.

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.

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.

64. Martín, 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.

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 Ercc1 -/Δ7 mice. Immun. Ageing 2019, 16, 6.

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.

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.

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.

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.

70. Szebeni, B.; Veres, G.; Dezsofi, A.; Rusai, K.; Vannay, A.; Bokodi, G.; Vásárhelyi, B.; Korponay-Szabó, I.R.; Tulassay, T.; Arató, A. Increased mucosal expression of Toll-like receptor (TLR)2 and TLR4 in coeliac disease. J. Pediatr. Gastroenterol. Nutr. 2007, 45, 187–193.

71. Kalliomäki, M.; Satokari, R.; Lähteenoja, H.; Vähämiko, S.; Grönlund, J.; Routi, T.; Salminen, S. Expression of microbiota, Toll-like receptors, and their regulators in the small intestinal mucosa in celiac disease. J. Pediatr. Gastroenterol. Nutr. 2012, 54, 727–732.

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.

73. Hooper, L.V.; Littman, D.R.; Macpherson, A.J. Interactions between the microbiota and the immune system. Science 2012, 336, 1268–1273.

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.

75. Schilderink, R.; Verseijden, C.; De Jonge, W.J. Dietary inhibitors of histone deacetylases in intestinal immunity and homeostasis. Front. Immunol. 2013, 4, 226.

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.

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.

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.

80. Palma, G.D.; Capilla, A.; Nova, E.; Castillejo, G.; Varea, V.; Pozo, T.; Garrote, J.A.; Polanco, I.; López, A.; Ribes-Koninckx, 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.

81. Medina, M.; De Palma, G.; Ribes-Koninckx, 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.

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.

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.

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.

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.

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

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.

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.

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.

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.

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.

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.

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 paracasei CBA L74 on bacterial-induced cellular imbalance. Cell. Microbiol. 2019, 21, e13035.

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.

95. Galipeau, H.J.; McCarville, J.L.; Huebener, S.; Litwin, O.; Meisel, M.; 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.

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.

97. De Palma, G.; Kamanova, J.; Cinova, 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.

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.

99. Laparra, J.M.; Sanz, Y. Bifidobacteria inhibit the inflammatory response induced by gliadins in intestinal epithelial cells via modifications of toxic peptide generation during digestion. J. Cell. Biochem. 2010, 109, 801–807.

100. 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.

101. Papista, C.; Gerakopoulos, V.; Kourelis, A.; Sounidaki, M.; Kontana, A.; Berthelot, L.; Moura, I.C.; Monteiro, R.C.; Yiangou, M. Gluten induces coeliac-like disease in sensitised mice involving IgA, CD71 and transglutaminase 2 interactions that are prevented by probiotics. Lab. Investig. 2012, 92, 625–635.

102. Fernandez-Feo, M.; Wei, G.; Blumenkranz, G.; Dewhirst, F.E.; Schuppan, D.; Oppenheim, F.G.; Helmerhorst, E.J. The cultivable human oral gluten-degrading microbiome and its potential implications in coeliac disease and gluten sensitivity. Clin. Microbiol. Infect. 2013, 19, E386–E394.

103. König, J.; Brummer, R.J. Is an enzyme supplement for celiac disease finally on the cards? Expert Rev. Gastroenterol. Hepatol. 2018, 12, 531–533.

104. Valitutti, F.; Trovato, C.M.; Montuori, M.; Cucchiara, S. Pediatric Celiac Disease: Follow-Up in the Spotlight. Adv. Nutr. 2017, 8, 356–361.

105. Norsa, L.; Tomba, C.; Agostoni, C.; Branchi, F.; Bardella, M.T.; Roncoroni, L.; Conte, D.; Elli, L. Gluten-free diet or alternative therapy: A survey on what parents of celiac children want. Int. J. Food Sci. Nutr. 2015, 66, 590–594.