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Salivary and fecal microbiota and metabolome of celiac children under gluten-free diet

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A B S T R A C T

Celiac disease (CD) is an inflammatory autoimmune disorder resulting from the combination of genetic predisposition and gluten ingestion. A life-long gluten free diet (GFD) is the only therapeutic approach. Dysbiosis, which can precede the CD pathogenesis and/or persist when subjects are on GFD, is reviewed and discussed. Salivary microbiota and metabolome differed between healthy and celiac children treated under GFD (T-CD) for at least two years. The type of GFD (African- vs Italian-style) modified the microbiota and metabolome of Saharawi T-CD children. Different studies showed bacterial dysbiosis at duodenal and/or fecal level of patients with active untreated CD (U-CD) and T-CD compared to healthy subjects. The ratio of protective anti-inflammatory bacteria such as *Lactobacillus-Bifidobacterium* to potentially harmful *Bacteroides-Enterobacteriaceae* was the lowest in U-CD and T-CD children. In agreement with dysbiosis, serum, fecal and urinary metabolome from U-CD and T-CD patients showed altered levels of free amino acids and volatile organic compounds. However, consensus across studies defining specific bacteria and metabolites in U-CD or T-CD patients is still lacking.

Future research efforts are required to determine the relationships between CD and oral and intestinal microbiotas to improve the composition of GFD for restoring the gut dysbiosis as a preventative or therapeutic approach for CD.

Keywords:

Celiac disease

Microbiota

Metabolome

Gluten-free diet

1. Introduction

Celiac disease (CD) is an autoimmune condition secondary to an immunological response to ingested gluten, in genetically susceptible individuals. CD is characterized by the presence of a variable combination of gluten-dependent clinical manifestations, CD-specific antibodies (anti-transglutaminase IgA, anti-endomysial-IgA and anti-deamidated gliadin-IgA), HLA-DQ2 or HLA-DQ8 haplotypes, and enteropathy. CD is triggered by the ingestion of gliadin and related prolamins (wheat, barley and rye) that are rich in glutamine and proline residues and resist to mammalian protease digestion, creating toxic immunogenic peptides. These peptides have glutamine residues that are preferably susceptible to deamination by tissue transglutaminase (tTG). This modification makes the peptides recognizable by HLA-DQ2 molecule of antigen-presenting cells (DQ2+ or DQ8+). In turn, DQ2+ or DQ8+ cells submit gluten peptides to CD4+ T cells that trigger the T-helper-cell type-1 response, with the consequent, gamma interferon-mediated, development of celiac lesion. The latter is represented by intraepithelial and

lamina propria infiltration of inflammatory cells, crypt hyperplasia, and villous atrophy (Stepniak and Koning, 2006).

CD was thought to affect exclusively Europeans. However, it is distributed worldwide, affecting 0.6 to 1.0% of the world's population (Fasano and Catassi, 2012; Lionetti et al., 1999; Stepniak and Koning, 2006). Epidemiological studies in Africa, Middle East, Asia, and South America have shown that CD is present but mainly underdiagnosed, with areas of higher prevalence, such as the Saharawi population in North Africa where the highest prevalence of CD (5.6%) known in the world today is registered (Teresi et al., 2010). CD commonly appears in early childhood, with typical symptoms including chronic diarrhea, abdominal distension, and failure to thrive or atypical symptoms such as short stature, iron deficiency anemia, hypertransaminasemia, oral ulcers or enamel defect. However, symptoms may not develop until later in life, when the disease occurs with fatigue, weight loss, anemia, neurological symptoms and or complications (auto-immune conditions). CD is a life-long disorder and, if untreated, is associated with increased morbidity and mortality (Di Sabatino and Corazza, 2009).

Once the diagnosis is achieved, the therapeutic approach is a strict, life-long gluten free diet (GFD). However, complete exclusion of gluten is difficult due to the ubiquitous nature of this protein and cross-contamination of foods. Selected sourdough biotechnology applied for the

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production of gluten-free leavened products could be useful to eliminate gluten contamination (Di Cagno et al., 2008; Gobetti et al., 2014). Barley glutamine-specific endoprotease, prolyl-endopeptidase from *Sphingomonas capsulata*, peptidase from selected sourdough lactic acid bacteria, prolyl-endoprotease from *Aspergillus niger* showed the capacity to digest gluten under gastrointestinal conditions (Mitea et al., 2008; De Angelis et al., 2010; Caputo et al., 2010). Indeed, alternative therapeutic options could be the oral supplementation of oligopeptidases to hydrolyze gluten immunogenic peptides.

CD commonly appears in children after the first exposures to gluten, but an increasing number of patients is also experiencing CD onset in late adulthood or adult age, suggesting that additional environmental factors could play a role in CD development (Cenit et al., 2015). An increasing number of evidences strongly indicate that oral and intestinal microbiotas play a central role in human health and disease (Claesson et al., 2012; Ling et al., 2013; Petersen, 2003; Zarco et al., 2012). A condition of dysbiosis is associated with CD patients at the diagnosis but also in remission (e.g., after two years of GFD) (for reviews see Cenit et al., 2015; Francavilla et al., 2014; Marasco et al., 2016; Verdu et al., 2015).

It was suggested that intestinal dysbiosis is strongly correlated with the pathogenesis and progression of several gastro-intestinal diseases such as dyspepsia, diarrhea, Inflammatory bowel disease (IBD), colorectal cancer (CRC), CD, and Irritable bowel syndrome (IBS) (Rautava et al., 2012). Genetic predisposition and environmental factors (e.g., diet, appendectomy, and antibiotic use) could be involved in the intestinal dysbiosis. Subjects at risk of developing CD show an association between altered microbiota and genes, which encode factors for bacterial sensing, immune reaction and metabolism. HLA-DQ2 and -DQ8 genotypes, therefore, select for pathobionts at an early stage, before the clinical onset of CD. Such an altered microbial composition also induces down-regulation of genes, which usually mark the healthy balance (Galipeau et al., 2015; Verdu et al., 2015).

Current data suggest that diet is a major driver of the composition and function of intestinal microbiota and could serve as a means of therapeutic intervention for prevention of diseases (Sonnenburg and Bäckhed, 2016; Voreades et al., 2014; Albenberg and Wu, 2014; Flint et al., 2015; Jeffery and O'Toole, 2013). GFD, per se, may affect the composition of gut microbiota and metabolome (Baranska et al., 2013; Sanz, 2010). Among different dietary components, fiber is associated to positive effects on gut microbiota and related metabolome (De Angelis et al., 2015; Sonnenburg et al., 2016; Vitaglione et al., 2015). Fibers are notably reduced in the Western diet (high in fat and simple carbohydrates, low in fibers), compared to Mediterranean diet. Overall, GFD is characterized by lower content of dietary fibers and resistant starch, compared to standard gluten-containing diet (De Palma et al., 2009; Kinsey et al., 2008; Miranda et al., 2014). Compared to a standard gluten-containing diet, healthy subjects being on GFD showed lower level of *Lactobacillus-Bifidobacterium*, along with higher number of *Enterobacteriaceae* (De Palma et al., 2009; Golfetto et al., 2014; Uy et al., 2015). In addition, GFD decreased the production of pro-inflammatory cytokines and chemokines (TNF- α , interferon- γ and IL-8) and anti-inflammatory cytokines (IL-10) by peripheral blood mononuclear cells (De Palma et al., 2009). The dysbiosis and the modification of the immunological properties suggest that CD children under GFD (T-CD) may lead to increased health risks in T-CD (Sanz, 2010; Galipeau et al., 2015).

Based on the key-role played by gut microbiota in human health, this review aims at describing the most recent advances about oral and fecal microbiota and metabolome of celiac children under GFD.

2. Salivary microbiota and metabolome in T-CD children

Human salivary microbiota is composed of ca. 700 bacterial species, reaching cell density of ca. 11 log CFU/g of wet weight dental plaque and 8–9 log CFU/g of saliva (Maukonen et al., 2008). Several bacterial species are involved in oral diseases, such as dental caries and periodontitis (Bik

et al., 2010; Ling et al., 2010). In addition, non-oral diseases such as bacterial endocarditis (Lockhart and Durak, 1999), heart disease (Beck et al., 2005), obesity (Piombino et al., 2014), pneumonia (Paju and Scannapeico, 2007), atherosclerosis (Koren et al., 2011) and preterm low birth weight (Boggess et al., 2006) are also correlated with several oral bacteria. Saliva has recently been considered as a new tool for diagnosis of some diseases (Zarco et al., 2012).

2.1. The salivary microbiota associated to T-CD children

Compared to healthy individuals (HC), the salivary microbiota of T-CD children is different (Acar et al., 2012; Ercolini et al., 2015; Francavilla et al., 2014). Based on culture-dependent methods, T-CD children were characterized by the lowest prevalence of salivary mutans streptococci and lactobacilli (Acar et al., 2012) and total anaerobes, and by an increased level of *Enterobacteriaceae* (Francavilla et al., 2014). Analyzing community-level catabolic profiles, the lowest values of Shannon's diversity and substrate richness were found in T-CD children (Francavilla et al., 2014). Consistently, 16S rRNA gene-based metagenetics data showed the lowest values of richness estimator (Chao1) and diversity index (Shannon) in the saliva of T-CD children. The relative abundance of several operational taxonomic units (OTUs) differed between the salivary samples of T-CD children and those of HC. Within Firmicutes, *Lachnospiraceae*, *Gemellaceae*, and *Streptococcus sanguinis* were mainly associated with T-CD children. *Streptococcus thermophilus* markedly decreased in T-CD children compared to HC. Other Firmicutes (e.g., *Veillonella parvula*), associated with oral health (Kumar et al., 2005), were found at the lowest relative amount in the saliva of T-CD (Francavilla et al., 2014). Compared to HC, T-CD children showed a decreased level of Bacteroidetes (such as *Porphyromonas* sp., *Porphyromonas endodontalis*, and *Prevotella nanceiensis*), and a lower amount of Actinobacteria, *Actinomyces*, *Atopobium* and *Corynebacterium durum*. *Rothia mucilaginosa*, involved in gluten degradation (Fernandez-Feo et al., 2013; Zamakhchari et al., 2011), was the only Actinobacteria species higher in T-CD compared to HC children (Francavilla et al., 2014).

Recently, the salivary microbiota of fourteen T-CD Saharawi children, with biopsy-confirmed CD, and being on an African-style diet was studied (Ercolini et al., 2015). Saharawi T-CD children showed alpha-diversity indexes similar to the Italian T-CD children. However, Saharawi T-CD children showed an unusually high level of some Firmicutes (*Clostridium*, *Eubacterium*, *Mogibacterium*, *Catonella*, *Peptococcus*, *Filifactor*, *Peptostreptococcus*), Actinobacteria (*Actinomyces*, *Rothia*) and Tenericutes (*Bulleidia*) compared to Italian T-CD and HC children (Fig. 1) (Ercolini et al., 2015; Francavilla et al., 2014).

A characterization of the salivary microbiota of U-CD patients at the diagnosis is needed to understand the role of the salivary microbiota in the etiology of CD. Studies dealing with different severities of CD may be useful to determine the correlations between the severity of CD and the salivary microbiome.

2.2. Diet drives the salivary microbiota of T-CD children

In a recent attempt to understand the effect of westernization, Saharawi T-CD children were moved to Italy and subjected, for two months, to shift from the traditional African-style GFD to the Italian-style GFD (Ercolini et al., 2015). Compared to Italian-style GFD, African-style diet was richer in gluten-free cereals, legumes and vegetables, with a lower intake of animal proteins, sugars, starch and fat. Compared to African-style GFD, the relative abundance of *Granulicatella*, *Capnocytophaga*, *Porphyromonas* and *Neisseria* was higher after 30 and, especially, 60 days of Italian-style GFD, altering the 'salivary type' of the individuals (Fig. 1). As shown by partitioning around medoid (PAM) clustering, using the relative abundance of core genera, Italian HC and T-CD and Saharawi T-CD children salivary samples were grouped into three "salivary types" (Ercolini et al., 2015). The salivary

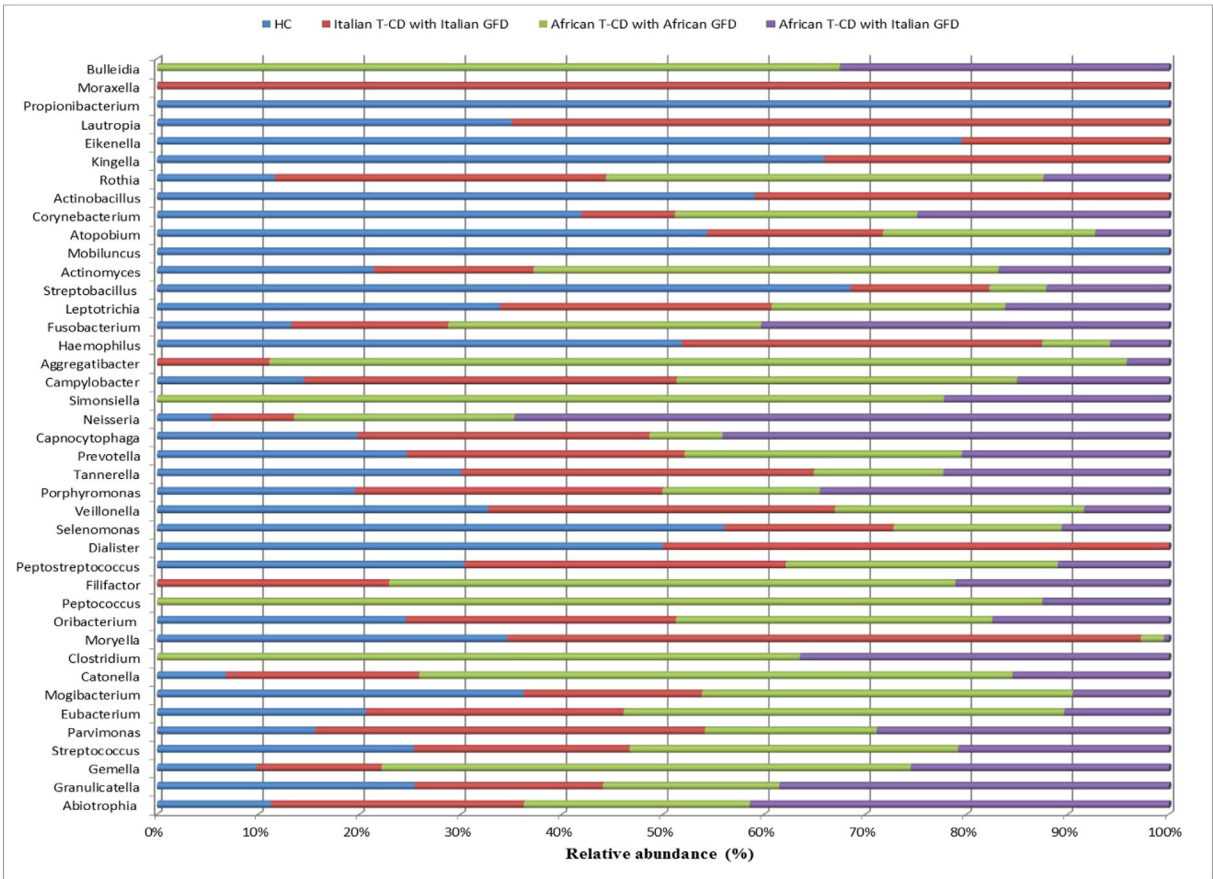


Fig. 1. Relative proportions (percent) of genera showing significant ($P < 0.05$) differences between the saliva samples of treated celiac (T-CD) children and those of healthy children. HC, Italian healthy children; Italian T-CD with Italian GFD, Italian T-CD children under Italian-style gluten-free diet (GFD); African T-CD with African GFD, African T-CD children with African-style GFD; and African T-CD with Italian GFD, African T-CD children with Italian-style GFD. The relative abundance of each genus was showed as percentage within the three groups of children.

samples of Saharawi T-CD children were grouped in the clusters II (mainly grouping samples collected after 30 and 60 days of Italian-style GFD) and III (mainly grouping samples collected during African-style GFD), which were associated to the highest amount of *Porphyromonas*, *Neisseria* and *Granulicatella* and *Streptococcus*, *Actinomyces*, *Peptococcus* and *Clostridium*, respectively. OTU co-occurrence/exclusion patterns showed that the initial equilibrium of co-occurring microbial species was modified by a change in diet, leading to a decrease of the microbial diversity, with a few OTUs out-competing the previously established microbiota and becoming dominant. The abundance of several OTUs (e.g., gluten-degrading *Clostridium* and *Rothia* species) can be attributed to environmental and dietary factors, as suggested by the decrease of those OTUs following the period wherein the Saharawi children were on Italian-style GFD. Several correlations were found between OTUs and dietary components. *Veillonella*, *Streptococcus*, *Catonella*, *Mogibacterium*, *Clostridium*, *Peptococcus*, *Gemella* and Actinobacteria showed positive correlations with dietary components (e.g., fiber) mainly abundant in the African-style GFD. Bacteroidetes, *Porphyromonas*, and *Capnocytophaga* were negatively correlated with carbohydrates and fibre intake and positively correlated with proteins, iron, calcium, phosphorus, caloric intake and/or lipids. A similar trend was also found for *Neisseria* and *Granulicatella* (Ercolini et al., 2015).

Compared to HC, saliva samples of U-CD and T-CD patients showed a significant difference in buffer capacity, IgA levels, minute volume, calcium and Ca/P ratio and protein composition (Mina et al., 2008, 2012). This different composition of the saliva in T-CD patients could modify the oral environment and, consequently, the composition and activity of the microbiota.

2.3. Salivary metabolome associated to T-CD children

Salivary metabolome was analyzed for different human healthy aspects: physiology, diagnostics, functional genomics, pharmacology, toxicology, and nutrition (Winder et al., 2011; Zhang et al., 2012). The salivary metabolome is originated by compounds from different origins, such as environmental exposure through inhalation, food intake, transdermal absorption and metabolic activities of oral microbiota. Regarding the latter, the metabolic products by microbiota interact with human cells (Want et al., 2007) and may also affect the total metabolomic profile (AL-Kateb et al., 2013). Habitual diet was suggested to have an influence on the composition of organic metabolites found in the saliva of healthy adults (De Filippis et al., 2014), again highlighting that diet has a pivotal role in the oral physiology even in healthy individuals. Recently, saliva metabolome was studied in T-CD children (Francavilla et al., 2014; Ercolini et al., 2015). According to the composition of the microbiota, the level of salivary volatile organic compounds (VOC) differed between T-CD and HC children (Fig. 2) (Francavilla et al., 2014). The level of several alcohols and phenols (e.g., 2-ethyl-1-hexanol, 4-1,1,3,3-tetramethylbutyl-phenol, and ethyl alcohol), hydrocarbons (e.g., 1-octadecene), ketones, terpenes, butanoic acid, acetic acid ethyl ester and octanal were the highest in salivary samples of HC. On the contrary, nonanal, 2-methyloctyl ester, carbone disulfide, halogenated and aromatic hydrocarbons (e.g., 1-chlorodecane and trichloromethane) were associated to T-CD children. Previously, increased levels of 2-propyl-1-pentanol, nonanal, dihydro-4-methyl-2(3H)-furanone and nonanoic acid were found in breath of HC subjects under GFD, compared to gluten-containing diet (Baranska et al., 2013).

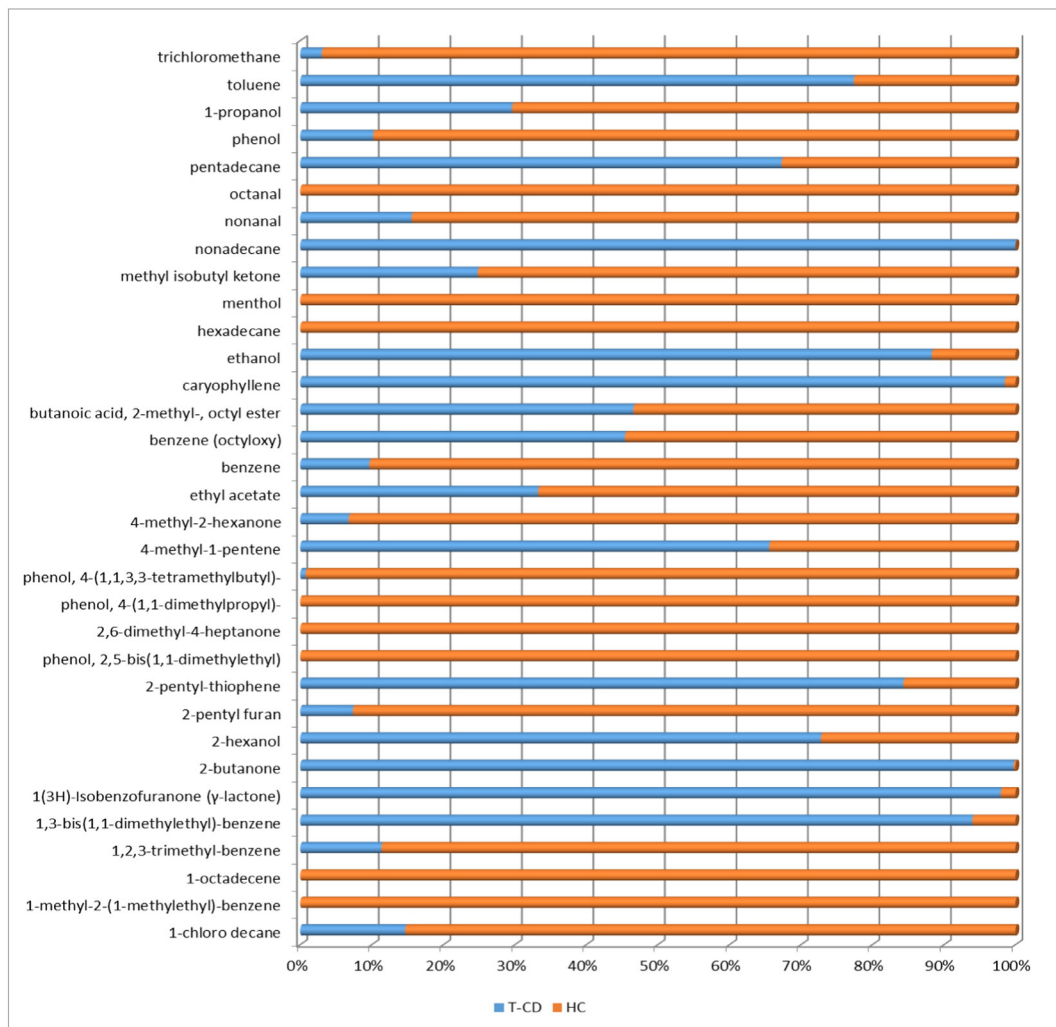


Fig. 2. Relative proportions (percent) of volatile organic compounds showing significant ($P < 0.05$) differences between the saliva samples of treated celiac (T-CD) children and those of healthy children (HC). The relative abundance of each compound was showed as percentage within the three groups of children.

Salivary microbiota and VOC were variously correlated (Francavilla et al., 2014). In detail, *Prevotella* sp. was positively correlated with the levels of nonanal and 1-chlorodecane. *C. durum* was positively associated to the level of 1,2,3-trimethylbenzene, 2,6-dimethyl-4-heptanone, 4-methyl-2-hexanone, and 4-methyl-3-penten-2-one. *G. adiacens*, *Atopobium* sp., and Bacilli and *V. parvula*, *S. thermophilus*, and the division SR1 showed correlations with the amounts of 1(3H)-isobenzofuranone (-lactone) and 1-octadecene, and γ -lactone and 1-chlorodecane, respectively. The above data support the hypothesis that the composition of the oral metabolome can be affected by the GFD and composition of the microbiome. The salivary metabolome of Saharawi T-CD children strongly differed from Italian HC and T-CD children (Fig. 3). Several VOC, such as 1-propanol, 4-(1,1,3,3-tetramethylbutyl)-phenol and phenol, heptanal, octanal and nonanal, 2-pentyl furan 1,3-bis 1,1-dimethylethyl-benzene; 1-chlorodecane; 1-octadecene, benzene and trichloromethane, strongly decreased in Saharawi celiac children during Italian-style GFD. At the same time, the amount of other VOC (e.g., acetone, 2-butanone and 3-methylbutanone) increased under Italian-style GFD. Analysis (by PICRUSt) of predicted metagenomes of the salivary microbiome of the Saharawi T-CD children showed a remarkable modification of the metabolic potential of the microbiome following the diet change, with increased amino acid, vitamin and co-factor metabolisms during Italian-style GFD (Ercolini et al., 2015). In addition, several positive and negative

correlations were detected between the relative abundances of several OTUs and VOCs (Fig. S1). For instance, *Actinomyces*, showing the highest level in saliva samples of Saharawi T-CD on an African-style GFD, showed positive correlations with VOC detected at 0 day of Italian-style GFD and negative correlations with other VOC detected at 60 days of Italian-style GFD (Fig. S1 panel T0 vs T60). Other OTUs that were dominant during African-style GFD showed negative correlation with VOC associated to Italian-style GFD (e.g., *Clostridium* versus furan, and *Eubacterium* versus ethyl acetate) (Fig. S1 panel T30). Salivary metabolome of African T-CD children was also affected by the dietary components of GFD (Fig. S2). For instance, furans, acetone, ethyl acetate, 2-butanone and 3-methyl-2-butanone were positively correlated with the intake of proteins. As expected, acetone was also strongly associated to the intake of lipids. Based on this data, dietary components and the initial salivary microbiota drive the salivary metabolome, which, in turn, modifies the colonization and balance of other bacteria (Ercolini et al., 2015).

All together the above data showed that CD patients have a different salivary microbiota and, especially, metabolome compared to those of healthy control. The limitation of the above studies was related to the small number of CD patients analyzed. However, the findings of these studies support the need of more extensive analyses to define how salivary microbiota/metabolome could impact CD or whether it is just a marker of health status in these individuals.

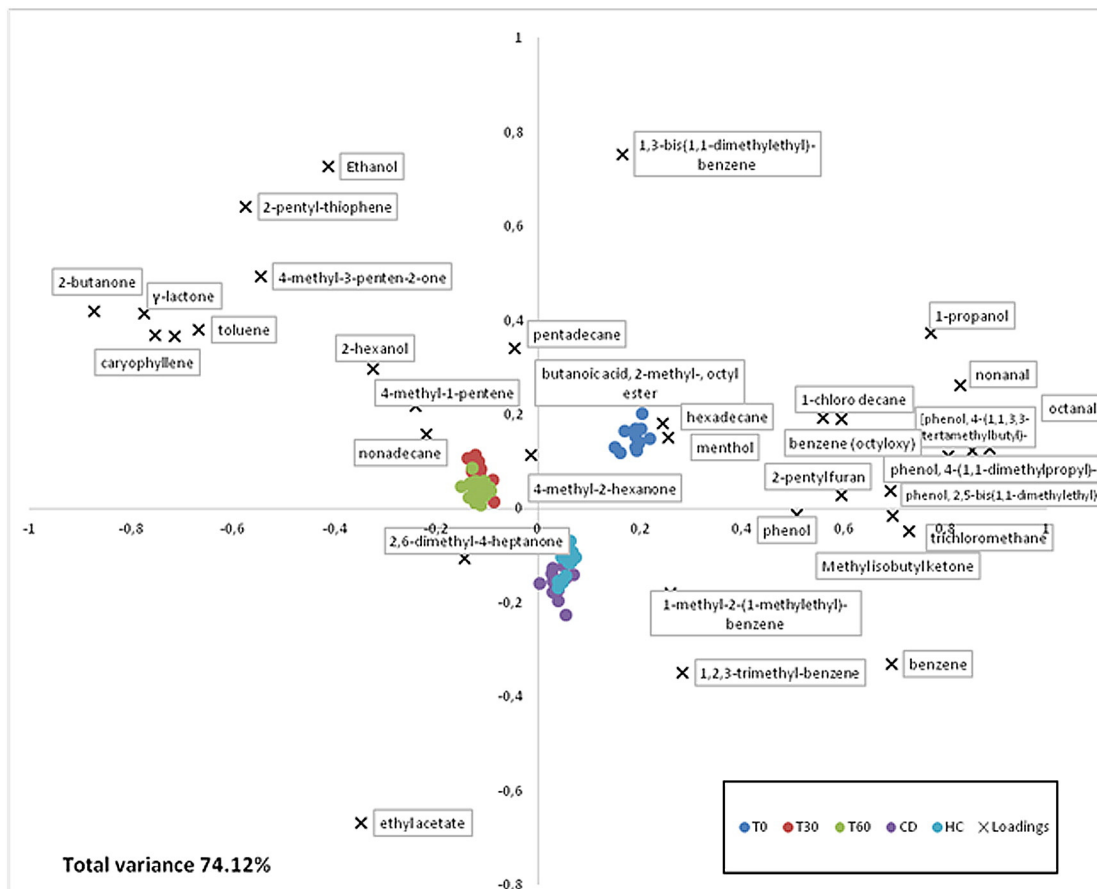


Fig. 3. Canonical discriminant analysis of principal coordinates of volatile organic compounds showing significant ($P < 0.05$) differences between the saliva samples of treated celiac (T-CD) children and those of healthy children. T0, African T-CD children with African-style gluten-free diet (GFD); T30, African T-CD children after 30 days of treatment with Italian-style GFD; T60, African T-CD children after 60 days of treatment with Italian-style GFD; CD, Italian T-CD children under Italian-style GFD; HC, Italian healthy children under gluten-content diet.

3. Intestinal microbiota and metabolome of T-CD children

The intestinal tract is the broadest body-environment interface, where various external materials, such as food components (including allergens) and bacteria (including pathogens), interact with the human body (Verdu et al., 2015). The human intestinal microbiota start gut colonization from the birth and reach levels up to 12 log of bacteria/g of colon (Zoetendal et al., 2012). Bacteria have intimate relationship with the human gut, conditioning the healthy or diseased state by metabolic activities and affecting nutrient absorption, barrier functions and immune development. The integrity of the intestinal microbiota is essential for maintaining a healthy state (Schipa and Conte, 2014). Apart from gluten, additional environmental factors that affect the CD pathogenesis could involve specific bacterial components (Verdu et al., 2015).

3.1. Intestinal microbiota associated to T-CD children

Different studies showed bacterial dysbiosis at duodenal and/or fecal level of CD patients with active untreated (U-CD; celiac subjects under gluten-containing diet) or treated (T-CD; celiac subjects under GFD for at least two years) disease, compared to healthy subjects (Fig. 4). Duodenal biopsy specimens of U-CD or T-CD children showed the highest abundance of Gram-negative bacteria (Collado et al., 2009; Di Cagno et al., 2011; Nadal et al., 2007; Nistal et al., 2012a; Sánchez et al., 2010, 2013; Schippa et al., 2010). Compared to HC, duodenal biopsies of U-CD showed higher levels of *Neisseria*, *Streptococcus*, *Serratia* and *Haemophilus* (Cheng et al., 2013; Ou et al., 2009). T-CD patients with persistent CD symptoms showed higher levels of Proteobacteria

(e.g., *Actinobacter* and *Neisseria*) compared to HC (Wacklin et al., 2014). Overall, the GFD treatment did not completely restore the normal intestinal microbiota (for reviews see de Sousa Moraes et al., 2014; Marasco et al., 2016; Verdu et al., 2015). The relative abundance of *Bacteroides*, *Escherichia coli*, *Enterobacteriaceae*, and *Staphylococcus* groups was higher in the U-CD than in the healthy subjects (Collado et al., 2009; Nadal et al., 2007; Sánchez et al., 2010, 2013). The ratio of *Lactobacillus-Bifidobacterium*, and *Bacteroides-Enterobacteriaceae* was lower in U-CD and T-CD compared to healthy subjects (Di Cagno et al., 2011; Nadal et al., 2007; Schippa et al., 2010). In addition, different balance of *Bifidobacterium* species was found in the duodenal biopsies of U-CD and T-CD compared to HC (Collado et al., 2008). The amount of some *Staphylococcus* and *Enterococcus* species also differed in fecal samples of U-CD and T-CD compared to HC (Sánchez et al., 2012a). The relative amount of *Bacteroides* was higher in U-CD and/or T-CD than in healthy children (Collado et al., 2007, 2009; Di Cagno et al., 2011; Nadal et al., 2007; Sánchez et al., 2010; Schippa et al., 2010). However, *Bacteroides* is a dominant genus of human gut microbiota and only some species (e.g., *B. vulgatus*, *B. fragilis*) show pro-inflammatory effect (Setoyama et al., 2003), suggesting the importance of identification at the species level (de Sousa Moraes et al., 2014; Sánchez et al., 2012b). Compared to healthy subjects, the relative abundance of *Bacteroides-Prevotella* increased in U-CD and T-CD children, along with a reduction of IgA-coated bacteria, suggesting the existence of mucosal barrier defects in CD patients (De Palma et al., 2010a). The gut microbiota could affect the pathogenesis and progression of CD by activation of innate immune system, modulation of the epithelial barrier and/or exacerbation of the immune response to gliadin peptides (Verdu et al., 2015; Galipeau et al., 2015).

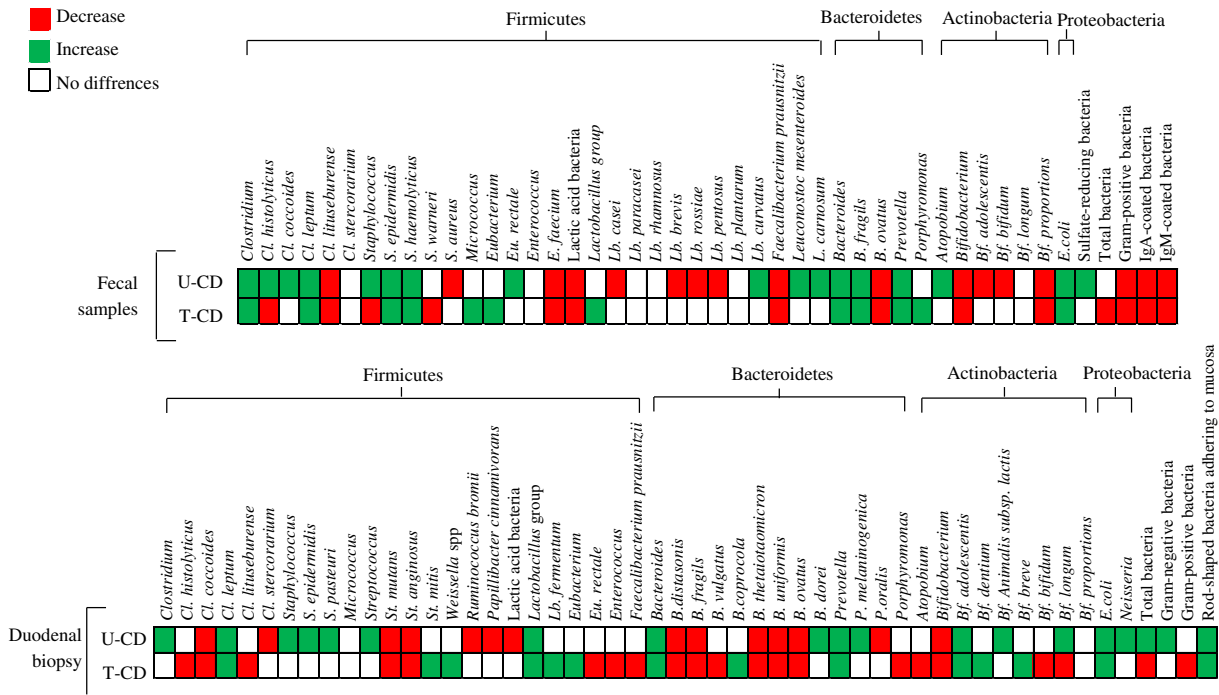


Fig. 4. Summary of alterations related to fecal and duodenal-associated microbiota in children with untreated (U-CD) and treated (T-CD) celiac disease compared to healthy controls. *Lb.*, *Lactobacillus*; *L.*, *Leuconostoc*; *B.*, *Bacteroides*; *Bf.*, *Bifidobacterium*; *E.*, *Eubacterium*; *En.*, *Enterococcus*; *Cl.*, *Clostridium*; *S.*, *Staphylococcus*; *F.*, *Faecalibacterium*; *St.*, *Streptococcus*, *P.*, *Prevotella*.

Various studies suggested that genetics and gut microbiota play a role in the susceptibility to CD. Compared to infants with low genetic risk of CD, *Bacteroides-Prevotella* group, Gram-negative bacteria, *Streptococcus-Lactococcus*, *Eubacterium rectale*, *Clostridium coccoides*, *Clostridium lituseburense*, *Clostridium histolyticum* and sulfite-reducing bacteria were higher in infants with high genetic risk of CD (De Palma et al., 2010a, 2010b; Olivares et al., 2014). High level of *Bacteroides* was found also in infants with high genetic risk of CD (Sánchez et al., 2011). In addition, a study with large cohort of infants showed low level of *Bifidobacterium* and increase of *Bacteroides fragilis* and *Staphylococcus* associated with infants with high genetic risk of CD (de Palma et al., 2012).

However, consensus across studies to define specific bacterial genera and/or species in active CD or T-CD patients is still lacking (Verdu et al., 2015). Several variables and confounding factors (e.g., age, methodology, sampling technique, length of GFD and clinical conditions) make the comparison of different studies hard. Although CD is common to children and adults (Vivas et al., 2008), only few studies are available for the intestinal composition of U-CD and T-CD adults (Nistal et al., 2012a, 2012b). To determine the exact relationship between gut microbiota and CD, further studies including CD adults are necessary (de Sousa Moraes et al., 2014; Verdu et al., 2015).

3.2. Serum, fecal and urinary metabolome associated to T-CD children

Compared to healthy controls, U-CD and T-CD patients showed unbalanced serum, fecal and urine metabolome (Di Cagno et al., 2009, 2011; Bertini et al., 2009). U-CD patients showed the lowest levels of serum amino acids, lipids, pyruvate and choline, and the highest levels of glucose and 3-hydroxybutyric acid (Bertini et al., 2009). The modified levels of glucose and ketones suggest alterations of energy metabolism in U-CD patients. After 12 months of GFD, an improved glucose metabolism, a decrease of 3-hydroxybutyric acid and an increase of methionine, valine, lactate and creatinine were found in the urine (Bertini et al., 2009). In agreement with gut dysbiosis, urines from U-CD and T-CD patients showed altered levels of some microbial metabolites,

such as indoxyl sulfate, meta-[hydroxyphenyl]propionic acid and phenylacetylglucine (Bertini et al., 2009). Median values of Lys, Arg, creatine and methylamine were higher in T-CD than in HC children (Di Cagno et al., 2011). On the contrary, carnosine, glucose, glutamine and 3-methyl-2-oxobutanoic acid were the highest in HC children. In addition, fecal levels of Trp, Pro, Asn, His, Met, trimethylamine-N-ox and tyramine were higher in T-CD than HC children. The level of total esters in fecal and urine samples was higher in HC compared to T-CD children, but several of them (ethyl-acetate, octyl-acetate, propyl-butyrate, propyl-propanoate and butyl 2-methylbitanoate) were the highest in fecal samples of T-CD (Di Cagno et al., 2011). Esterification reactions in colon could be the microbial strategy to detoxify acids or alcohols (Vitali et al., 2010). Aldehydes were higher in HC children than in T-CD (Di Cagno et al., 2011). According to salivary metabolome, some alcohols (e.g., 1-octen-3-ol, ethanol and 1-propanol) were found at the highest level in T-CD. Ethanol synthesized by intestinal bacteria was correlated with non-alcoholic steatohepatitis (NASH) (Cani et al., 2008), which is associated to occult CD (Grieco et al., 2001). Short chain fatty acids (SCFA) (e.g., isocaproic, acetic, butyric and propionic acids) differed in fecal samples of U-CD and T-CD children compared to healthy children (Di Cagno et al., 2009, 2011; Tjellström et al., 2005). The altered composition of the metabolome of U-CD and T-CD subjects could be related to CD, as well as to bacterial dysbiosis and composition of GFD. Several bacterial metabolites (e.g., acetic acid, propionic acid and butyric acid) and structures (e.g., polysaccharide A of *B. fragilis*) could modify the immune responses of CD patients (Verdu et al., 2015).

It is widely accepted that microbial metabolites probably act as mediators for the host metabolism with positive (e.g., butyrate) or detrimental (e.g., trimethylamine-N-ox) effects (Sonnenburg and Bäckhed, 2016). In the same time, the production of VOCs and other metabolites is affected by complex interactions between host, composition of microbiota and diet. Consequently, meta-genomics, meta-transcriptomic and/or meta-proteomics studies with the reconstruction of metabolic pathways could be the best tool to elucidate the relationship between microbiota and metaboloma, microbiota and immune function, and metaboloma and immune function in CD patients.

4. Concluding remarks and future perspectives

Although not yet exhaustive, the current literature clearly shows the association between CD, gut microbiota and metabolome. Altered gut microbiota and metabolome could play a secondary role in aggravating CD pathogenesis or other diseases in celiac patients by modifying the host immunity and physiology. CD patients at the diagnosis, when still consuming a gluten-containing diet, showed different microbiota compared to healthy controls, suggesting that the microbiota is involved in the CD pathogenesis (Cenit et al., 2015). Another hypothesis is that CD can promote dysbiosis, which, in turn, can increase the inflammation by pro-inflammatory pathobionts (e.g. strains belonging to *Enterobacteriaceae*, *B. fragilis*) and decline of anti-inflammatory mutualistic bacteria (e.g. strains belonging to *Lactobacillus* and *Bifidobacterium*). Further studies linking bacterial dysbiosis and development of CD in high-risk infants will help to define the gene-microbe interactions and the role of the microbiota on the CD pathogenesis and progression (Verdu et al., 2015; Galipeau et al., 2015).

However, consensus across studies defining specific bacteria and metabolites in children and adult celiac patients is still lacking. In addition, GFD could also affect the gut microbiota and, consequently, the metabolome of celiac patients (De Palma et al., 2009; Bonder et al., 2016). To date, studies were performed only comparing the microbiota and metabolome of T-CD patients under GFD with those of healthy volunteers under gluten-containing diet. Wheat is the major source of fructans in the diet, and contains 1–4% fructans on solid matter (Gibson and Shepherd, 2010). Intake of prebiotic fructans (oligofructose and inulin) resistant starch and dextrin from cereals during gluten-containing diet promotes growth of anti-inflammatory mutualistic bacteria (Slavin, 2013). Further studies are strongly recommended to elucidate the effect exerted by certain compounds contained in cereals on the gut microbiota. This could be done by comparing T-CD patients to healthy subjects under GFD.

Data on the use of probiotics (e.g., bifidobacteria) for CD are encouraging, but to date only a few studies (de Sousa Moraes et al., 2014) have been conducted on humans, also with contradictory results. For probiotics, the complexity of the microbiome and the intra and inter-subject variability further complicate the definition of what a desired state may look like for a population or for a given individual. By contrast, a growing interest is assuming the quality and preparation of gluten-free foods. Emerging dietary treatments are not only economical, but also offer a targetable and non-invasive approach for chronic states of inflammation. After long-time work, our group developed a biotechnology strategy aimed at hydrolyzing gluten (<8 ppm of residual concentration) during food processing with a combination of selected sourdough lactobacilli and food-grade fungal proteases (Greco et al., 2011). Conceptually, the only dietary difference between CD and healthy individuals (gluten or similar protein-containing flours) was eliminated. In conclusion, dietary strategies could improve the intestinal microbiota and host immunity increasing the resilience to CD.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.ijfoodmicro.2016.07.025>.

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