



Review

Non-coeliac gluten sensitivity: A review of the literature

Joana Clímaco Henggeler^a, Manuel Veríssimo^b, Fernando Ramos^{a, c, *}^a Pharmacy Faculty, University of Coimbra, Azinhaga de Santa Comba, 3000-548 Coimbra, Portugal^b Serviço de Medicina, Centro Hospitalar e Universitário de Coimbra, Medicine Faculty, University of Coimbra, Praceta Mota Pinto, 3000-075 Coimbra, Portugal^c CNC – Center for Neuroscience and Cell Biology, Pharmacy Faculty, University of Coimbra, Azinhaga de Santa Comba, 3000-548 Coimbra, Portugal

ARTICLE INFO

Article history:

Received 27 August 2016

Received in revised form

23 May 2017

Accepted 28 May 2017

Available online 1 June 2017

Keywords:

Coeliac disease

Gluten

Gluten-free diet

Irritable bowel syndrome

Non-coeliac gluten sensitivity

Wheat allergy

ABSTRACT

Background: Non-coeliac gluten sensitivity (NCGS) is an emerging and still poorly defined clinical entity, which is part of the spectrum of gluten-related disorders (along with coeliac disease and wheat allergy) but also closely related to irritable bowel syndrome. It is characterized by a wide array of both gastro-intestinal and extra-intestinal symptoms. NCGS was first defined in the 1970s, but has gained critical relevance in recent years.

Scope and approach: This review covers the existing definitions, documented symptoms and methods of diagnosis, treatment via a gluten-free diet, history, prevalence in the general population and possible mechanisms for NCGS. It also looks into recent studies and their findings. All information is relative to studies published in the last 6 years (2011–present).

Key findings and conclusions: Despite recent advances in characterising NCGS as a clinical entity and gaining novel insights into its pathogenesis, further studies are needed to better understand its etiology and mechanism and to establish specific biomarkers.

© 2017 Elsevier Ltd. All rights reserved.

1. Gluten and the spectrum of gluten-related disorders

Gluten is the main storage protein complex in cereals such as wheat, barley, rye and spelt. Its main protein constituents are gliadin and glutenin (Dupont, Vensel, Tanaka, Hurkman, & Altenbach, 2011). These proteins are rich in glutamines and prolamines, which causes them to be incompletely digested by gastric, pancreatic, and brush border peptidases, leading to the formation of large peptides (Shan et al., 2002). These pass through the intestinal epithelial barrier and enter the lamina propria by way of a transcellular or paracellular route (Visser, Rozing, Sapone, Lammers, & Fasano, 2009).

When flours which contain gluten are kneaded with water, the dough acquires viscosity and elasticity due to the action of gliadins and glutenins (Blomfeldt, Kuktaite, Johansson, & Hedenqvist, 2011). This happens by way of the formation of an elastic network which retains the gases resulting from fermentation, which allows the expansion and rise of the dough during the baking process (Moore, Schober, Dockery, & Arendt, 2004).

The proteins of gluten possess resistance to gastric and intestinal digestion and cause an increase in intestinal permeability through cytoskeletal rearrangement, zonulin (protein which modulates intestinal permeability) overexpression and dysfunction of tight junctions (Drago et al., 2006). Intestinal homeostasis is altered via the inhibition of epithelial cell growth and the induction of apoptosis (Dolfini et al., 2005).

The ingestion of gluten can trigger an array of conditions; these are designated by the broader term “gluten-related disorders”. They are divided into: disorders with autoimmune pathogenesis, including coeliac disease (CD); disorders characterized by allergic mechanisms, which include wheat allergy (WA); and the controversial non-coeliac gluten sensitivity (NCGS), whose causes are neither autoimmune nor allergic in nature (Sapone et al., 2012).

1.1. Coeliac disease

Coeliac disease (CD) is a systemic immune-mediated disorder caused by gluten and analogous prolamines (found in rye and barley) in individuals with a genetic susceptibility (Sapone et al., 2012). It is considered to affect between 1 and 2% of the general population (DiGiacomo, Tennyson, Green, & Demmer, 2013; Sapone et al., 2012).

* Corresponding author. Pharmacy Faculty, University of Coimbra, Azinhaga de Santa Comba, 3000-548 Coimbra, Portugal.

E-mail addresses: fjramos@ci.uc.pt, framos@ff.uc.pt (F. Ramos).

Abbreviations

AGA	anti-gliadin antibodies	IBS	inflammatory/irritable bowel syndrome
ASD	autism spectrum disorders	IBS-D	diarrhoea-predominant inflammatory/irritable bowel syndrome
ATI(s)	amylase trypsin inhibitor(s)	IFN- γ	interferon-gamma
CCL2	chemokine (C-C motif) ligand 2	IgA, IgE, IgG	immunoglobulins A, E and G
CD	coeliac disease	IL-8	interleukin-8
CD3, CD4, CD14	clusters of differentiation 3, 4 and 14	LBP	lipopolysaccharide-binding protein
CLDN1, CLDN4	claudins 1 and 4	LPS	lipopolysaccharide
DBPC	double-blind placebo-controlled	MD2	lymphocyte antigen 96
DBPCFC	double-blind placebo-controlled food challenge	NCGI	non-coeliac gluten intolerance
DQ2, DQ8	human major histocompatibility class II alleles	NCGS	non-coeliac gluten sensitivity
EMA	anti-endomysium autoantibodies	NCWS	non-coeliac wheat sensitivity
FABP2	fatty acid-binding protein 2	RCD	refractory coeliac disease
FODMAPs	fermentable oligo-, di-, and mono-saccharides and polyols	sCD14	soluble CD14
FOXP3	forkhead box P3	TG2	transglutaminase 2
GFD	gluten-free diet	TGF	transforming growth factor
GI	gastrointestinal	TLR-1, TLR-2, TLR-4	Toll-like receptors –1, –2 and –4
GS	gluten sensitivity/gluten sensitive	T _{REG}	regulatory T-cell
HLA	human leukocyte antigen	tTG	tissue transglutaminase
		WA	wheat allergy

CD possesses well-documented genetic makeup and environmental triggers (such as gliadin peptides) (Sapone et al., 2010). Markers used in the confirmation of a diagnosis are IgA tissue transglutaminase (tTG or TG2) and anti-endomysial (EMA) antibodies (Sapone et al., 2010, 2011; Sollid & Jabri, 2011).

The common feature for all patients suffering from CD is the presence of a variable combination of gluten-dependent clinical manifestations, specific antibodies (tTG/TG2, EMA), human leukocyte antigen (HLA)-DQ2 and/or -DQ8 haplotypes (present in 90–95% of CD patients) and degrees of enteropathy varying from lymphocytic infiltration of the epithelium to complete villous atrophy (Sollid & Jabri, 2011).

The immune response to gluten in this disorder involves both the adaptive and innate immune systems (though the former is more heavily implicated in this mechanism) (Sollid, 2002). An adaptive immune reaction takes place within the lamina propria. Gliadin is deamidated by tTG, and the latter increases its immunogenicity by altering the charge of the gliadin fragments that aid in the binding between antigen presenting cells and the HLA-DQ2 or -DQ8 molecule (Sollid, 2002). This reaction leads to inflammatory responses in the small intestine that are mediated by CD4⁺ T cells (Sollid, 2002). Said cells recognize gliadin peptides, leading to the subsequent production of pro-inflammatory cytokines, specifically interferon gamma (IFN- γ) (Sollid, 2002). The release of metalloproteinases and other tissue damaging mediators concurrent with the inflammatory cascade induce tissue injury (Mohamed et al., 2006).

Diagnosis is based on clinical symptoms (mainly chronic diarrhoea and weight loss), histopathological evaluation of biopsies of the small intestine with varying degrees of villous atrophy, as well as serological detection of TG2 and/or EMA IgA antibodies (Sollid & Jabri, 2011).

Until recently, CD was thought to be uncommon during infancy or childhood, while also being detected more frequently in individuals of that age (Matthias et al., 2011). However, it is now recognized that the majority of CD cases occur in adults in the 40–60 years age range (Matthias et al., 2011). This disorder is also more common in females than in males (Volta, Caio, Tovoli, & De Giorgio, 2013). First-degree family members of CD patients

have been found to possess an increased risk for CD, which can vary from 2 to 20% depending on gender and HLA-haplotype (Vriezinga et al., 2014).

The foundation of treatment for CD is the permanent adherence to a strict gluten-free diet (GFD), which will usually lead to a rapid improvement, both clinical and histological (Green & Cellier, 2007). This treatment calls for the exclusion of wheat and other cereal grains from the diet (Green & Cellier, 2007). Patients on a GFD will have a quick response in most cases, leading to a resolution of clinical symptoms. Nonetheless, the resolution of histological changes is a longer process (Sestak & Fortgang, 2013).

Some patients will, however, develop refractory forms of CD (RCD), being unable to respond to the GFD and maintaining an inflammation. Repeated dietary transgressions and continuing gluten consumption (e.g., in non-diagnosed individuals) are thought to be contributing factors to the development of RCD (Hadithi & Peña, 2010).

Finally, regarding the association of CD and the risk of malignant lymphomas, a gluten-free diet does not appear to alter the risk of lymphoma. However, it is not possible to completely eliminate a cause-effect relationship (Olén et al., 2011). The risk of occurrence of malignant lymphoma in CD seems to be related with the small intestine histopathology, without raising the risk of latent CD (Elfström et al., 2011).

More recently, Lebwohl et al. (2013) found that the risk is mainly related with the T cell lymphoma, without a high level risk of the B cell lymphoma, and conclude that the follow-up biopsy may be a means to effectively stratify CD patients regarding subsequent malignant lymphoma risks.

1.2. Wheat allergy

Wheat allergy (WA) is an adverse immunological reaction to gluten and other proteins found in wheat (National Institute of Allergy and Infectious Diseases, 2010; Sapone et al., 2012). As it is mediated by IgE, the pathogenesis of CD and WA are believed to be unrelated, in spite of their optimal treatments being the same (Sestak & Fortgang, 2013).

In WA, the ingestion of wheat causes typical IgE-mediated reactions of immediate onset, whose symptoms include: swelling and itching of the lips or mouth, urticaria, angioedema, bronchial obstruction, nausea and abdominal pain, or, in severe cases, systemic anaphylaxis (Domínguez-Ortega et al., 2014; National Institute of Allergy and Infectious Diseases, 2010). Late-onset manifestations appear around 24 h after the ingestion of wheat, including gastrointestinal (GI) symptoms (such as bloating, diarrhoea, vomiting and constipation) and aggravation of atopic dermatitis (Domínguez-Ortega et al., 2014; National Institute of Allergy and Infectious Diseases, 2010). Additional manifestations include fatigue, weight loss, joint pains and headaches, and, in rarer cases, eosinophilic esophagitis (Domínguez-Ortega et al., 2014). Sensitization to wheat proteins can be proved via the measurement of circulating IgE-specific antibodies against the suspected allergen and by skin sensitivity testing (which can be done either by prick or patch technique or by intradermal injection) (Mowszet, Matusiewicz, & Iwańczak, 2014).

2. Non-coeliac gluten-sensitivity

Non-coeliac gluten sensitivity (NCGS), also known as gluten sensitivity (GS), non-coeliac gluten intolerance (NCGI) and non-coeliac wheat sensitivity (NCWS) has been an intriguing and poorly defined entity for decades (Di Sabatino & Corazza, 2012; Sapone et al., 2012).

There is no officially established definition of NCGS (DiGiacomo et al., 2013). Some studies have defined it as a reaction to gluten in which allergic and autoimmune mechanisms have been excluded, but ingestion of foods containing wheat, rye and barley still lead to one or more of a wide range of immunological, morphological and symptomatic manifestations (Sapone et al., 2012). However, it must be said is not certain that this condition is triggered by gluten at all, with other components of wheat and other gluten-containing grains being possibly responsible, as detailed in later sections (Biesiekierski et al., 2013; Catassi et al., 2013; Gibson, Muir, & Newnham, 2015).

NCGS patients are traditionally defined as individuals who develop symptoms (which can be GI and similar to those of CD or irritable/inflammatory bowel syndrome (IBS), or extra-intestinal) after the ingestion of gluten-containing food but do not have definite evidence of CD or WA (Biesiekierski, Newnham, Shepherd, Muir, & Gibson, 2014; Catassi et al., 2013; Volta et al., 2013).

Said symptoms seem to develop after the ingestion of gluten-containing food, improve (or disappear altogether) when it is withdrawn from the diet and reappear when it is reintroduced (Sapone et al., 2012).

2.1. Symptoms

The time interval between the ingestion of gluten and the advent of symptoms in NCGS can vary from several hours to several days (Sapone et al., 2012; Volta et al., 2013). The majority of patients with NCGS will already have been suffering from these symptoms for months or even years by the time a diagnosis is reached (Volta et al., 2012).

NCGS incorporates a wide array of GI symptoms, such as abdominal discomfort and pain, bloating, aerophagia, flatulence, bowel habit abnormalities (either constipation or diarrhoea), dissatisfaction with stool consistency and gastroesophageal reflux (Di Sabatino & Corazza, 2012; National Institute of Allergy and Infectious Diseases, 2010; National Institute of Diabetes and Digestive and Kidney Diseases, 2008; Sapone et al., 2012; Volta et al., 2013).

Its extra-intestinal symptoms include headaches, nausea, “foggy

mind”/“brain fog”, anxiety, depression, blurry vision, fatigue, lethargy, musculoskeletal pains, dermatitis (eczema or skin rash), paraesthesia of the legs, arms, hands and feet, anaemia and attention-deficit/hyperactivity disorder (Carroccio et al., 2012; Catassi et al., 2013; Di Sabatino & Corazza, 2012; DiGiacomo et al., 2013; Sapone et al., 2012).

More severe neurologic and psychiatric conditions including schizophrenia, “idiopathic” cerebellar ataxia, neuropathies, epilepsy, mood swings, and autism spectrum disorders (ASD) have also been claimed to be associated with NCGS (Canales, Mery, Larrondo, Bravo, & Godoy, 2006; Dickerson et al., 2010; Hadjivassiliou et al., 2010; Ludvigsson, Reichenberg, Hultman, & Murray, 2013).

2.2. Diagnosis

The diagnosis is mostly based on an association between the ingestion of gluten-containing grains and the development of adverse symptoms, once CD and WA have been ruled out and the patient has started a GFD (Sapone et al., 2012).

NCGS patients do not suffer from the lesions in the duodenal mucosa that are characteristic of CD, but can in some cases present lymphocytic enteritis (Rosinach et al., 2016; Rostami et al., 2015; Volta et al., 2013). The recurrence of symptoms after the reintroduction of gluten to the diet has been proposed as a final step in the diagnostic process of NCGS (Volta & De Giorgio, 2012).

Currently, there are no specific biomarkers for this condition, neither in the form of antibodies or proteins or peptides in serum, nor of histological changes in the small intestine or elsewhere (Catassi et al., 2013; Sapone et al., 2012).

In the diagnostic process, CD should be eliminated by means of negative serology (IgA anti-tTG and anti-EMA) (Sapone et al., 2012; Volta et al., 2013). A duodenal biopsy is also strongly recommended due to the possibility of seronegative or “silent” CD (Volta & Villanacci, 2011). Patients presenting borderline serology should undergo HLA typing to determine the need for biopsy (Kabbani et al., 2014).

IgA-anti-gliadin antibodies (AGA), found in the vast majority of CD patients, are present in about half of NCGS patients (Volta et al., 2012). Even taking this into account, AGA might not be sufficiently adequate to be used as markers for a diagnosis of NCGS (Infantino et al., 2015).

WA should be ruled out through negative testing for specific IgE and/or prick tests (Mowszet et al., 2014; Sapone et al., 2012).

At present, NCGS is seen as a diagnosis of exclusion (Kabbani et al., 2014; Sapone et al., 2012). The suspected overlap between IBS and GS raises the need for even more stringent diagnostic criteria (Catassi et al., 2013; Volta et al., 2013).

Excluding gluten from the diet causes significant improvement, which includes the disappearance of both GI and extra-intestinal symptoms; reintroducing gluten causes the recurrence of symptoms (Catassi et al., 2013; Sapone et al., 2012; Volta & De Giorgio, 2012). The improvement or cessation of symptoms, along with their reappearance, attributable to the absence or presence of dietary gluten, suggests the presence of NCGS (Volta et al., 2013).

Nonetheless, double-blind, placebo-controlled (DBPC) challenge trials are actively recommended for the confirmation of the NCGS diagnosis, since a placebo effect induced by gluten withdrawal cannot be excluded (Lillie et al., 2011). A significant number of patients often refuse to return to a diet containing gluten if they have already independently started a GFD and have experienced symptom relief (which could lead to missed or at least delayed diagnoses) (Leffler, 2010). It is also necessary to consider that self-reporting of symptoms can be inaccurate, and as such, there will likely be a considerable difference in perceived versus actual NCGS

(Biesiekierski et al., 2014). The constitution of a proper gluten challenge is also an area where there is little agreement, since modalities and amounts of gluten used in clinical trials have been varied (Biesiekierski et al., 2011a; Carroccio et al., 2012). In addition, there is a risk of positive results based only on chance, with some studies therefore defending that the double-blind placebo-controlled food challenge (DBPCFC) should be repeated at least twice to minimize this risk (Husby & Murray, 2015).

The DBPCFC is not without limitations, however, as it has been linked to false-positive results and to a nocebo effect by reason of the issues associated with the measurement of subjective symptoms (Godlee, 2012). More recently, a review article has raised concerns about the adequacy of the DBPC gluten challenge as the gold standard for the confirmation of a NCGS diagnosis (Molina-Infante & Carroccio, 2017).

2.3. Gluten-free diet and its issues

The recommended treatment after a diagnosis of NCGS, as in the case of CD and WA, is a GFD (Pietzak, 2012). Patients must make the necessary alterations to their dietary habits and consume foods with minimal gluten content (less than 10 mg/day) (Volta et al., 2013). Cereals such as rice, corn, buckwheat, millet and teff and leguminosae such as quinoa, amaranth and soybean are recommended as substitutes for gluten-containing products (Foschia, Horstmann, Arendt, & Zannini, 2016; Volta et al., 2013). Many naturally gluten-free starches that can complement the GFD with important nutrients also exist; these include sweet potatoes, butternut or acorn squashes, beans, and lentils (Theethira & Dennis, 2015).

Gluten-free products which are commercially available are useful in helping NCGS patients achieve a fully gluten-free diet; however, naturally gluten-free foods (such as meat, fish, eggs, fruit and vegetables) should still be integrated into their diets to provide proper nutrition (Volta et al., 2013). The overall use of commercially available gluten-free products ought to remain low to limit the introduction of chemical additives and preservatives abundant in these products, which are a potential cause of functional GI symptoms (Volta et al., 2013).

It has to be said that the tolerance level to gluten varies across individuals, with some NCGS patients not being able to tolerate even minimal amounts of gluten (Volta et al., 2013). Most NCGS patients will experience the complete disappearance of symptoms on a GFD, while in some cases this improvement will only be partial (Volta et al., 2013).

Moreover, it should be considered that in the absence of a confirmed diagnosis of CD or NCGS, a GFD is no healthier than an analogous gluten-containing diet (Kulai & Rashid, 2014). Actually, the implementation of a GFD in the full absence of symptoms might only result in an increased cost with no benefit (Burden et al., 2015). In the absence of the adequate knowledge or supervision by a dietician and the necessary nutritional substitutions, the levels of fibre, vitamins (specifically B vitamins and folic acid) and minerals (such as iron, zinc, phosphorus, magnesium and calcium) can be too low and those of saturated fat and calories too high, originating nutritional deficiencies (Theethira & Dennis, 2015).

Long-term adherence to a GFD with a lack of nutritional supervision can even cause the development of obesity, new-onset insulin resistance and metabolic syndrome as the worst scenario (Kabani et al., 2012; Tortora et al., 2015). There is also emerging evidence that a GFD which is not sufficiently diverse may cause a greater risk of exposure to certain toxins (such as mycotoxins, heavy metals and pesticides) when compared to a diet containing gluten (Pellegrini & Agostoni, 2015).

Additionally, there is the issue of the increased costs of gluten-

free food products, leading to many patients finding a GFD unsatisfactory (Burden et al., 2015). Social isolation is also a concern with this diet, particularly in settings such as communal celebrations, religious rituals, and dining out (Simsek, Baysoy, Gencoglan, & Uluca, 2015). Furthermore, much like in the case of coeliac patients, rigorous avoidance of gluten can prove challenging due to its many hidden sources (Thompson & Simpson, 2015).

Future progress in the proper characterization of the factors related to CD and non-CD pathogenesis might aid in the development of new treatment strategies alternative to the GFD (Plugis & Khosla, 2015).

2.4. History

The existence of NCGS was first hypothesised in 1978 in a case report of a patient suffering from diarrhoea and intermittent abdominal pain, lacking abnormalities on a biopsy and experiencing improvement on a GFD (Ellis & Linaker, 1978).

This was followed by a double-blind crossover trial in 8 adult female patients who suffered from abdominal pain and chronic diarrhoea (in the absence of CD and WA) and had startling relief on a GFD, with the symptoms reappearing after a gluten challenge. All patients subsequently decided to remain on a GFD, having been on it for 4–6 years by the time of the report (Cooper et al., 1980).

Possibly due to a lack of a proper definition and specific tests for this condition and the fact its symptoms had an overlap with those of IBS, interest ceased (Holmes, 2013).

After more than two decades with no mention of NCGS, in 2000, Kaukinen and colleagues reported that 63% of 94 adults who complained of abdominal symptoms after gluten ingestion did not comply to the diagnostic criteria for CD and WA; as they also benefited from a GFD, they were considered to suffer from NCGS (Kaukinen et al., 2000).

A subsequent scarcity of data followed until 2007, when Wahnschaffe and co-workers described a group of patients with diarrhoea-predominant IBS (IBS-D) who showed clinical improvement on a GFD in the absence of CD. This response, however, was only significant in HLA-DQ2 positive patients who possessed IgG antibodies against tTG and gliadin (Wahnschaffe, Schulzke, Zeitze, & Ullrich, 2007).

The modern age of NCGS truly began in 2011, when Biesiekierski et al. performed the first randomized, DBPC, clinical trial in IBS patients with a history of GI symptoms kept in control by a GFD. This study decisively established the double-blind randomized controlled trial as feasible, which in turn led to its adoption as the optimal design of NCGS clinical studies. The success and influence garnered by this study stemmed from its appropriately simple primary aim (“to determine whether gluten ingestion can induce symptoms in non-coeliac individuals”) and design. Even though it was unable to reach a conclusion about the underlying mechanism, the study suggested gluten as a dietary trigger for symptoms (Biesiekierski et al., 2011a). More recently, in 2012, a revision of the nomenclature for gluten-related disorders was the first to officially include NCGS (Sapone et al., 2012).

2.5. Prevalence

The estimated prevalence of NCGS is widely variable in the literature, with cited figures ranging between 0.6 and 6% of the general population (this meaning it could be as high as six times that of CD) (DiGiacomo et al., 2013; Sapone et al., 2012).

In addition, it might be as high as 25–30% among IBS patients (Carroccio et al., 2012).

The clinical features of NCGS as determined from existing studies show that this disorder is rare, or at least uncommon, in

infancy and childhood (or possibly underdiagnosed in said age groups), being more frequent in adolescents and young to middle-aged adults, with a significant number of cases also diagnosed in the elderly (Catassi et al., 2013; Volta et al., 2012, 2013).

Similarly to CD, NCGS seems to be more common in females than in males (DiGiacomo et al., 2013; Volta & De Giorgio, 2012; Volta et al., 2012). It also seems to be more frequent in first-degree relatives of CD patients (Volta et al., 2012).

Importantly, it should be noted that the CD-predisposing HLA-DQ2 and -DQ8 genotypes are found in 50% of NCGS patients, a prevalence lower than in CD (95%) and only slightly higher than in the general population (30%) (Catassi et al., 2013; Sapone et al., 2012).

3. Possible mechanisms of non-coeliac gluten sensitivity

3.1. Innate or adaptive immune response

Many studies consider that NCGS has an enhanced innate immune response as its underlying mechanism, in contrast to the adaptive immunity seen in CD (Junker et al., 2012; Sapone et al., 2010, 2011).

Innate immunity relies on a receptor-mediated and immediate response to molecular patterns that are conserved and common in bacterial and viral antigens, and to some molecules that act as “danger” signals (Beutler, 2009).

Higher organisms are endowed with a diversity of innate, pathogen and danger signal recognizing receptors; these include the Toll-like receptors (TLRs), primarily expressed on myeloid cells (monocytes, macrophages and dendritic cells) (Beutler, 2009). It has been hypothesised that gluten and its related peptides may be the triggers, inducing the innate immune response and stimulating dendritic cells, which results in the infiltration of leukocytes and inflammation of the gut mucosa (Sapone et al., 2011).

It should be pointed out that the innate immune response is a first line of defence that is dependent on cytokines and does not provide long-lasting immunity, in contrast to the adaptive response seen in CD, which relies on antibody production and confers immunologic memory for future antigenic exposure (Sapone et al., 2011). The behaviour of the aforementioned TLRs (that play a key role in the innate immune system as well as the digestive system) gives support to the hypothesis of a stimulation of the innate immune system in NCGS that does not involve the IFN- γ pathway (Sapone et al., 2011).

TLRs are single, membrane-spanning, non-catalytic receptors usually expressed in sentinel cells (such as macrophages and dendritic cells). They recognize structurally conserved molecules derived from microbes (Sapone et al., 2011). When physical barriers, such as the skin or intestinal mucosa, are breached by said microbes, the latter are recognized by TLRs, which activate immune cell responses (Sapone et al., 2011).

The expression of TLR-2 (and, to a lesser extent, of TLR-1 and TLR-4) in the small intestine is greater in NCGS patients than in coeliacs (Sapone et al., 2011). The two disorders also differ in the small intestinal expression of the T-regulatory (T_{REG}) marker forkhead box P3 (FOXP3), considerably weaker in gluten-sensitive patients than in coeliacs (Sapone et al., 2010, 2011).

In vivo short-term gluten challenge of NCGS patients' mucosa has been observed not to cause alterations in the transcript levels of interleukin-8 (IL-8) and chemokine (C-C motif) ligand 2 (CCL2), two chemokines involved in the recruitment of immune cells in the inflamed gut (Brottveit et al., 2013).

An additional involvement of an adaptive immune response in NCGS has also been hypothesised (Brottveit et al., 2013). Said hypothesis is supported by observations of increased levels of IFN- γ

and density of intraepithelial CD3⁺ T-cells and decreased levels of transforming growth factor β 1 (TGF- β 1) and FOXP3 markers after a gluten challenge. These data indicate that there is a lower likelihood of recruitment of regulatory T lymphocytes to the small intestine in NCGS patients than in healthy individuals (Brottveit et al., 2013).

3.1.1. Amylase trypsin inhibitors

Amylase trypsin inhibitors (ATIs) are a family of homologous proteins of low molecular weight (Junker et al., 2012). They can be found in a variety of plants, where they inhibit enzymes of common parasites (such as mealworms and bugs) and are involved in regulating processes such as grain maturation and storage of carbohydrates (Dupont et al., 2011; Junker et al., 2012).

ATIs represent 2–4% of total wheat protein (in contrast to 80–90% for gluten) (Dupont et al., 2011). They are approximately 120–150 amino acids in length and display a compact secondary structure with 5 intramolecular disulphide bonds, which confer them a degree of resistance to proteolytic digestion by the GI proteases trypsin and pepsin (Junker et al., 2012).

These proteins have been shown to engage the TLR4–MD2–CD14 complex and lead to the upregulation of maturation markers, eliciting release of pro-inflammatory cytokines in cells from patients with and without CD and in coeliac patients' biopsies (Schuppan, Pickert, Ashfaq-Khan, & Zavallos, 2015). Consequently, after ingestion and during their passage through the intestine, ATIs preserve their biological activity of TLR-4 activation (Junker et al., 2012).

Members of the ATI family are strong activators of the innate immune responses of monocytes, macrophages and dendritic cells (Beutler, 2009; Junker et al., 2012). ATIs have also been previously characterized as allergens in baker's asthma and GI hypersensitivity to wheat (Junker et al., 2012). These observations have led to the hypothesis that ATIs might fuel inflammation and immune responses in other intestinal and non-intestinal immune disorders, such as CD and NCGS (Junker et al., 2012).

Of note, ATIs are present and even enriched in commercial gluten (Makharia, Catassi, & Makharia, 2015). Although they are not classified as gluten proteins, they are found in grain endosperm along with these, usually co-fractionating with the gliadin fraction (Makharia et al., 2015).

TLR-4 stimulating activity is mainly restricted to ATIs of gluten-containing grains (Junker et al., 2012; Schuppan et al., 2015). Therefore, plants other than wheat, rye, barley or their related ancestors show only minimal or absent TLR-4-activating activity, even though they contain (structurally different) inhibitors of amylase and trypsin-like activities (Junker et al., 2012; Schuppan et al., 2015). A GFD is, thus, also essentially ATI-free (Makharia et al., 2015; Schuppan et al., 2015). These findings prompted the suggestion that the term “non-coeliac wheat sensitivity” (NCWS) might be more appropriate than the previously established “NCGS” (Makharia et al., 2015; Schuppan et al., 2015).

The effect of ATIs is dose-dependent, and a significant reduction of their daily intake by a >90% avoidance of gluten-containing foods could be enough to decrease their effect of co-stimulation to an irrelevant level (Schuppan et al., 2015).

3.2. Gut dysbiosis

The microbiota, an integral part of both animal and human organisms, has a close inter-relationship with its host; it is an essential inherent factor that impacts human health (Daulatzai, 2015). Recent research has shown how changes in the composition of the gut microbiome, as well as microbial translocation, can influence animal and human physiology, contributing to a vast

array of diseases (Foster & McVey Neufeld, 2013).

An alteration in microbiome (dysbiosis) has thus been identified as the driving force behind development of diseases and dyshomeostasis (Daulatzai, 2015). Compromised integrity of the intestinal epithelium has been linked to extensive systemic immune responses, both innate and adaptive, resulting from translocation of microbes from the lumen into circulation (Estes et al., 2010).

Several studies point to the possibility of NCGS being a subset of IBS, rather than a gluten-related disorder (Fritscher-Ravens et al., 2014). This is mainly based on the overlap between the GI symptoms characteristic of both conditions, which could have a common mechanism (Di Sabatino & Corazza, 2012). Alterations in the gut microbiome have been speculated to be able to lead to an abnormal immune function in the gut, translating clinically into the classic IBS (and NCGS) symptoms (Wahnschaffe, Ullrich, Riecken, & Schulzke, 2001). It has also been hypothesised that the changes in gut microbiota possibly observed in IBS and NCGS might be caused by certain non-gluten components of wheat (Simrén et al., 2013). However, said alterations could also originate from a radical change in dietary habits (Simrén et al., 2013).

Dysbiosis may also be responsible for several of the extra-intestinal symptoms present in NCGS through the enhancement of gut inflammation, dysfunction of the intestinal barrier, and promotion of systemic-to-neuro-inflammation (Collins, Surette, & Bercik, 2012). It has also been linked to an accelerated development of CD through certain species of rod-shaped bacteria with the ability to colonise the human small intestine (Sjöberg et al., 2013). The regulation of microflora composition (e.g. by probiotics and prebiotics) offers the possibility to restore GI homeostasis and decrease neuro-inflammation by influencing the mucosal and systemic immunity dysfunction (Daulatzai, 2015).

Some alterations in gut microbiota were observed to have been restored to normal levels after adherence to a GFD (Sanz, De Pama, & Laparra, 2011). This suggests that these alterations are secondary consequences of the disease and possibly directly related to gluten consumption (Sanz et al., 2011).

3.3. Epithelial cell damage and systemic immune activation

It has been suggested that NCGS symptoms may result from an impaired barrier function of the intestinal mucosa (Sander, Cummins, Henshall, & Powell, 2005; Vogelsang, Schwarzenhofer, Steiner, Wyatt, & Oberhuber, 2001). It is thought that gluten induces malabsorption of various nutrients, leading to systemic deficiencies (Morant, 2011).

Various *in vitro* studies on cell cultures demonstrated that treatment with gluten can cause alterations in cellular morphology and motility, as well as in cytoskeleton organisation and intercellular contact through tight junction proteins (Dolfini et al., 2005; Roncoroni et al., 2013). Treated cells show a reduction in viability due to the stimulation of apoptosis and reduction of the synthesis of nucleic acids and proteins (Caputo et al., 2012).

CD patients have already been well-documented as having impaired intestinal permeability as a result of abnormal tight junction morphology (Vogelsang et al., 2001). Tight junctions are known to open in the presence of gluten, allowing exposure of luminal antigens within the lamina propria (Sander et al., 2005).

Claudins (CLDN) are protein components essential to the function of tight junctions (Sapone et al., 2011). When compared to CD patients, patients with NCGS have showed increased expression of CLDN4, which, along with CLDN1, is associated with normal tight junction function and intestinal permeability (Sapone et al., 2011).

Gluten having a role in mediating cholinergic activation is also a possibility (Biesiekierski et al., 2011a; Verdu et al., 2008). This could lead to increased smooth muscle contractility and have indirect

effects on luminal water content (Biesiekierski et al., 2011a; Verdu et al., 2008).

The hypothesis that the enhanced antibody response to native gliadin in NCGS/NCWS patients could be a consequence of ongoing defects in the intestinal epithelial barrier was advanced in a more recent study (Uhde et al., 2016). These defects would lead to an inadequate regulation of the interaction between the gut microbiota and systemic circulation, with peripheral immune activation as the result (Uhde et al., 2016). Translocated circulating lipopolysaccharides (LPS) can lead to the rapid secretion of lipopolysaccharide-binding protein (LBP) by GI and hepatic epithelial cells, as well as of soluble CD14 (sCD14) by CD14⁺ monocytes/macrophages (Branchley & Douek, 2012). The latter binds LPS in the presence of LBP to activate TLR-4 (Miller, Ernst, & Bader, 2005). The observed increased serum levels of sCD14 and LBP, elevated expression of fatty-acid binding protein 2 (FABP2) and antibody reactivity to microbial antigens indicated systemic immune activation and compromised integrity of the intestinal epithelial barrier (Uhde et al., 2016). This study also puts forward the possibility that these markers of systemic immune activation and epithelial cell damage might be of future use as potential biomarkers for the diagnosis of NCGS/NCWS, given that they are present in high concentrations in individuals suffering from this condition and decrease to normal levels after implementation of a diet excluding wheat and related cereals (Uhde et al., 2016).

3.4. Effect of non-protein components

Fermentable oligo-, di-, and mono-saccharides and polyols (FODMAPs) are poorly absorbed short-chain carbohydrates that cause distension of the intestinal lumen due to their small molecular size and rapid fermentability, originating functional GI symptoms (Gibson & Shepherd, 2012).

FODMAPs are found in a variety of foods, including those which contain lactose, fructose, fructans, galactans, and polyols (sorbitol, mannitol, and xylitol) (Gibson & Shepherd, 2012). Some common foods which are high in FODMAPs include wheat, rye and barley, as well as milk, honey, some fruits (e.g. apples, watermelons, cherries, mangos and pears) and vegetables (e.g. chicory, fennel, beetroot, leek, lentils and chickpeas) (Biesiekierski et al., 2011b; Volta et al., 2013).

These carbohydrates can trigger functional GI symptoms by inducing luminal distension through a combination of osmotic effects and production of gas, since they provide a substrate for rapid bacterial fermentation in the small and large intestine (Gibson & Shepherd, 2012; Gibson et al., 2015).

Many cereals which contain gluten are high in fructans, thus posing a problem for IBS patients, as their reduction accompanied by the introduction of a GFD may lead to an improvement in symptoms, wrongly perceived to result from a reduction in gluten intake (Biesiekierski et al., 2011b; Gibson & Shepherd, 2012).

A diet low in FODMAPs (containing, for instance, almond and rice milk, rice, oats and quinoa) has been observed to improve GI symptoms (such as abdominal pain, bloating, gas and diarrhoea) in NCGS patients (Halmos, Power, Shepherd, Gibson, & Muir, 2014). Despite this, a low-FODMAP diet is strict to follow and not intended to be a prolonged diet regimen (Öhman & Simrén, 2013).

In contrast, the role of FODMAPs in NCGS has been dismissed by some studies, which state that, although FODMAPs can cause certain GI symptoms, they inhibit, rather than cause, intestinal inflammation (Gibson et al., 2015). Moreover, they have also been shown to generate short-chain fatty acids and induce beneficial alterations in the intestinal microbiota (Makharia et al., 2015).

3.5. Placebo or nocebo effect

The profound effect of placebo has been well documented for a variety of functional GI disorders (Shah & Pimentel, 2014). The existence of a relevant placebo/nocebo phenomenon has actually been reported in DBPC trials in adult patients with self-reported food intolerances, and the possibility of gluten withdrawal resulting in a placebo effect caused has been pointed out (Godlee, 2012). Therefore, it is likely that a portion of patients with NCGS, and arguably a considerable one, fall in this category (Godlee, 2012).

In spite of all the recent studies, it is clear that NCGS is still a controversial issue (Di Sabatino & Corazza, 2012; Godlee, 2012). There is the definite probability that many patients present an imaginary syndrome with a subjective sensation of improvement resulting from a placebo effect or the withdrawal of gluten (Godlee, 2012). Many patients might be strongly influenced by the general popularity of the GFD as a healthy diet option (Di Sabatino & Corazza, 2012).

There is also the possibility that a nocebo effect could explain the current prevalence of NCGS to an extent, as patients believing themselves to be food sensitive would be preconditioned to avoidance (Biesiekierski et al., 2013). The results of a DBPC trial of gluten vs placebo demonstrate that gluten is the trigger of symptoms in a subgroup of patients fulfilling the diagnostic criteria for NCGS (Rosinach et al., 2016).

3.6. Other proposed mechanisms

3.6.1. Opioid activity of gluten

Recently, NCGS has been associated with the development of neuro-psychiatric disorders such as ASDs, schizophrenia and depression (Dickerson et al., 2010; Ludvigsson et al., 2013; Marcason, 2009).

The mechanism proposed as being responsible is a CD-unrelated, primary alteration of the small intestinal barrier ("leaky gut") that leads to an abnormal absorption of gluten peptides (resulting from incomplete breakdown of gluten) (Catassi et al., 2013; Marcason, 2009). Said peptides are able to eventually cross the intestinal membrane, enter the bloodstream, and cross the blood-brain barrier, affecting the endogenous opiate system and neurotransmission within the nervous system (Catassi et al., 2013). Gluten proteins can in fact mimic some of the effects of opiates by causing alterations in the intestinal transit of healthy volunteers in a naloxone-reversible manner (Corazza et al., 1984).

3.6.2. Food additives

Chemical additives such as glutamates, benzoates, sulphites and nitrates, added to many commercial products for a variety of reasons (for improvement of flavour, colour and preservative function), may have a role in eliciting the functional GI symptoms of NCGS and other conditions with characteristic intestinal inflammation, such as IBS (Gibson & Shepherd, 2012). Supporting this hypothesis is the fact that a subgroup of NCGS patients does not improve by eating commercially gluten-free products (rich in additives and preservatives), and only experience symptom resolution through implementation of a diet based on naturally gluten-free foods (Volta et al., 2013).

3.6.3. Early-stage coeliac disease

Even though NCGS and CD are in all likelihood two separate entities with distinct primary pathogenic pathways represented by innate and adaptive immunity, respectively, the probability that NCGS might evolve into CD cannot be discarded (Not et al., 2011; Volta et al., 2013).

The diagnosis of CD is clearly defined and follows a well-

standardized process, but instances where the disease cannot be recognized remain, as the process has yet to reach the point of being detectable (either by serum antibody testing or by pathology) (Picarelli et al., 2013). Therefore, it can be speculated that a fraction of patients labelled with NCGS may in fact suffer from seronegative CD and still lack overt intestinal damage (Picarelli et al., 2013).

The detection of deposits of IgA through small intestine biopsies may help in the identification of NCGS patients at risk of developing CD (Not et al., 2011; Volta & Villanacci, 2011). NCGS patients possessing HLA-DQ2/-DQ8 haplotypes actually suffering from CD is also a possibility (Mooney, Aziz, & Sanders, 2013).

4. Conclusion

Non-coeliac gluten sensitivity is still a poorly characterized clinical entity, in spite of recent advances. Its very definition varies between publications, and its pathogenesis is still the source of some controversy. It is still debated if NCGS is indeed part of the spectrum of gluten-related disorders and related to CD, or instead a subset of IBS. Likewise, it is unclear if its symptoms result from an innate immune response, intestinal dysbiosis, systemic immune activation, epithelial cell damage or other causes, or even if they are caused by gluten at all, as opposed to other component(s) of wheat. Some studies have put its very existence into doubt, favouring the existence of a potential placebo or nocebo effect as the cause of the presented symptoms.

It is clear that further studies are needed to gain insight on the etiology and potential biomarkers (perhaps specifically exploring the viability of FABP2 and LBP, among others, as suggested by the recent study by Uhde et al.) for this condition, so that its patients can have the answers and treatment they deserve.

References

- Beutler, B. (2009). Microbe sensing, positive feedback loops, and the pathogenesis of inflammatory diseases. *Immunological Reviews*, 227(1), 248–263.
- Biesiekierski, J. R., Newnham, E. D., Irving, P. M., Barrett, J. S., Haines, M., Doecke, J. D., et al. (2011a). Gluten causes gastrointestinal symptoms in subjects without celiac disease: A double-blind randomized placebo-controlled trial. *The American Journal of Gastroenterology*, 106(3), 508–514.
- Biesiekierski, J. R., Newnham, E. D., Shepherd, S. J., Muir, J. G., & Gibson, P. R. (2014). Characterization of adults with a self-diagnosis of nonceliac gluten sensitivity. *Nutrition in Clinical Practice: Official Publication of the American Society for Parenteral and Enteral Nutrition*, 29(4), 504–509.
- Biesiekierski, J. R., Peters, S. L., Newnham, E. D., Rosella, O., Muir, J. G., & Gibson, P. R. (2013). No effects of gluten in patients with self-reported non-celiac gluten sensitivity after dietary reduction of fermentable, poorly absorbed, short-chain carbohydrates. *Gastroenterology*, 145, 320–328.
- Biesiekierski, J. R., Rosella, O., Rose, R., Liels, K., Barrett, J. S., Shepherd, S. J., et al. (2011b). Quantification of fructans, galacto-oligosaccharides and other short-chain carbohydrates in processed grains and cereals. *Journal of Human Nutrition and Dietetics: The Official Journal of the British Dietetic Association*, 24(2), 154–176.
- Blomfeldt, T. O., Kuktaite, R., Johansson, E., & Hedenqvist, M. S. (2011). Mechanical properties and network structure of wheat gluten foams. *Biomacromolecules*, 12(5), 1707–1715.
- Brenchley, J. M., & Douek, D. C. (2012). Microbial translocation across the GI tract. *Annual Review of Immunology*, 30, 149–173.
- Brottveit, M., Beitnes, A. C., Tollefsen, S., Bratlie, J. E., Jahnsen, F. L., Johansen, F. E., et al. (2013). Mucosal cytokine response after short-term gluten challenge in celiac disease and non-celiac gluten sensitivity. *The American Journal of Gastroenterology*, 108(5), 842–850.
- Burden, M., Mooney, P. D., Blanshard, R. J., White, W. L., Cambray-Deakin, D. R., & Sanders, D. S. (2015). Cost and availability of gluten-free food in the UK: In store and online. *Postgraduate Medical Journal*, 91(1081), 622–626.
- Canales, P., Mery, V. P., Larrondo, F. J., Bravo, F. L., & Godoy, J. (2006). Epilepsy and celiac disease: Favorable outcome with a gluten-free diet in a patient refractory to antiepileptic drugs. *The Neurologist*, 12(6), 318–321.
- Caputo, I., Secondo, A., Lepretti, M., Paoletta, G., Auricchio, S., Barone, M. V., et al. (2012). Gliadin peptides induce tissue transglutaminase activation and ER-stress through Ca²⁺ mobilization in Caco-2 cells. *PLoS One*, 7(9), e45209.
- Carroccio, A., Mansueto, P., Iacono, G., Soresi, M., D'Alcamo, A., Cavataio, F., et al. (2012). Non-celiac wheat sensitivity diagnosed by double-blind placebo-controlled challenge: Exploring a new clinical entity. *The American Journal of*

- Gastroenterology, 107(12), 1898–1906.
- Catassi, C., Bai, J. C., Bonaz, B., Bouma, G., Calabrò, A., Carroccio, A., et al. (2013). Non-celiac gluten sensitivity: the new frontier of gluten related disorders. *Nutrients*, 5(10), 3839–3853.
- Collins, S. M., Surette, M., & Bercik, P. (2012). The interplay between the intestinal microbiota and the brain. *Nature Reviews. Microbiology*, 10(11), 735–742.
- Cooper, B. T., Holmes, G. K., Ferguson, R., Thompson, R. A., Allan, R. N., & Cooke, W. T. (1980). Gluten-sensitive diarrhea without evidence of celiac disease. *Gastroenterology*, 79(5 Pt 1), 801–806.
- Corazza, G. R., Frazzoni, M., Strocchi, A., Prati, C., Sarchielli, P., & Capelli, M. (1984). Alimentary exorphin actions on motility and hormonal secretion of gastrointestinal tract. In F. Fraioli, A. Isidori, & M. Mazzetti (Eds.), *Opioid peptides in the periphery* (pp. 243–247). Amsterdam: Elsevier Sciences Publisher.
- Daulatzai, M. A. (2015). Non-celiac gluten sensitivity triggers gut dysbiosis, neuroinflammation, gut-brain axis dysfunction, and vulnerability for dementia. *CNS & Neurological Disorders Drug Targets*, 14(1), 110–131.
- Di Sabatino, A., & Corazza, G. R. (2012). Non-celiac gluten sensitivity: Sense or sensibility? *Annals of Internal Medicine*, 156, 309–311.
- Dickerson, F., Stallings, C., Origoni, A., Vaughan, C., Khushalani, S., Leister, F., et al. (2010). Markers of gluten sensitivity and celiac disease in recent-onset psychosis and multi-episode schizophrenia. *Biological Psychiatry*, 68(1), 100–104.
- DiGiacomo, D. V., Tennyson, C. A., Green, P. H., & Demmer, R. T. (2013). Prevalence of gluten-free diet adherence among individuals without celiac disease in the USA: Results from the continuous national health and nutrition examination survey 2009–2010. *Scandinavian Journal of Gastroenterology*, 48(8), 921–925.
- Dolfini, E., Roncoroni, L., Elli, L., Fumagalli, C., Colombo, R., Ramponi, S., et al. (2005). Cytoskeleton reorganization and ultrastructural damage induced by gliadin in a three-dimensional in vitro model. *World Journal of Gastroenterology*, 11(48), 7597–7601.
- Domínguez-Ortega, G., Borrelli, O., Meyer, R., Dziubak, R., De Koker, C., Godwin, H., et al. (2014). Extraintestinal manifestations in children with gastrointestinal food allergy. *Journal of Pediatric Gastroenterology and Nutrition*, 59(2), 210–214.
- Drago, S., El Asmar, R., Di Pierro, M., Grazia Clemente, M., Tripathi, A., Saponi, A., et al. (2006). Gliadin, zonulin and gut permeability: Effects on celiac and non-celiac intestinal mucosa and intestinal cell lines. *Scandinavian Journal of Gastroenterology*, 41(4), 408–419.
- Dupont, F. M., Vensel, W. H., Tanaka, C. K., Hurkman, W. J., & Altenbach, S. B. (2011). Deciphering the complexities of the wheat flour proteome using quantitative two-dimensional electrophoresis, three proteases and tandem mass spectrometry. *Proteome Science*, 9, 10.
- Elfström, P., Granath, F., Ekström Smedby, K., Montgomery, S. M., Asklund, J., Ekblom, A., et al. (2011). Risk of lymphoproliferative malignancy in relation to small intestinal histopathology among patients with celiac disease. *Journal of the National Cancer Institute*, 103(5), 436–444.
- Ellis, A., & Linaker, B. D. (1978). Non-celiac gluten sensitivity? *Lancet*, 1(8078), 1358–1359.
- Estes, J. D., Harris, L. D., Klatt, N. R., Tabb, B., Pittaluga, S., Paiardini, M., et al. (2010). Damaged intestinal epithelial integrity linked to microbial translocation in pathogenic simian immunodeficiency virus infections. *PLoS Pathogens*, 6(8), e1001052.
- Foschia, M., Horstmann, S., Arendt, E. K., & Zannini, E. (2016). Nutritional therapy – facing the gap between coeliac disease and gluten-free food. *International Journal of Food Microbiology*, 239, 113–124.
- Foster, J. A., & McVey Neufeld, K. A. (2013). Gut-brain axis: How the microbiome influences anxiety and depression. *Trends in Neurosciences*, 36(5), 305–312.
- Fritscher-Ravens, A., Schuppan, D., Ellrichmann, M., Schoch, S., Röcken, C., Brasch, J., et al. (2014). Confocal endomicroscopy shows food-associated changes in the intestinal mucosa of patients with irritable bowel syndrome. *Gastroenterology*, 147(5), 1012–1020. e4.
- Gibson, P. R., Muir, J. G., & Newnham, E. D. (2015). Other Dietary Confounders: FODMAPS et al. *Digestive Diseases*, 33(2), 269–276.
- Gibson, P. R., & Shepherd, S. J. (2012). Food choice as a key management strategy for functional gastrointestinal symptoms. *The American Journal of Gastroenterology*, 107(5), 657–666.
- Godlee, F. (2012). Gluten sensitivity: Real or not? *British Medical Journal*, 345(1), e8450.
- Green, P. H., & Cellier, C. (2007). Celiac disease. *The New England Journal of Medicine*, 357(17), 1731–1743.
- Hadithi, M., & Peña, A. S. (2010). Current methods to diagnose the unresponsive and complicated forms of coeliac disease. *European Journal of Internal Medicine*, 21(4), 247–253.
- Hadjivassiliou, M., Sanders, D. S., Grünewald, R. A., Woodroffe, N., Boscolo, S., & Aeschlimann, D. (2010). Gluten sensitivity: From gut to brain. *The Lancet. Neurology*, 9(3), 318–330.
- Halmos, E. P., Power, V. A., Shepherd, S. J., Gibson, P. R., & Muir, J. G. (2014). A diet low in FODMAPs reduces symptoms of irritable bowel syndrome. *Gastroenterology*, 146(1), 67–75. e5.
- Holmes, G. (2013). Non coeliac gluten sensitivity. *Gastroenterology and Hepatology from Bed to Bench*, 6(3), 115–119.
- Husby, S., & Murray, J. (2015). *Non-celiac gluten hypersensitivity: What is all the fuss about? F1000Prime Reports* (Vol. 7), 54.
- Infantino, M., Manfredi, M., Meacci, F., Grossi, V., Severino, M., Benucci, M., et al. (2015). Diagnostic accuracy of anti-gliadin antibodies in non-celiac gluten sensitivity (NCGS) patients: A dual statistical approach. *Clinica Chimica Acta; International Journal of Clinical Chemistry*, 451(Pt B), 135–141.
- Junker, Y., Zeissig, S., Kim, S. J., Barisani, D., Wieser, H., Leffler, D. A., et al. (2012). Wheat amylase trypsin inhibitors drive intestinal inflammation via activation of toll-like receptor 4. *The Journal of Experimental Medicine*, 209(13), 2395–2408.
- Kabbani, T. A., Goldberg, A., Kelly, C. P., Pallav, K., Tariq, S., Peer, A., et al. (2012). Body mass index and the risk of obesity in coeliac disease treated with the gluten-free diet. *Alimentary Pharmacology & Therapeutics*, 35(6), 723–729.
- Kabbani, T. A., Vanga, R. R., Leffler, D. A., Villafuerte-Galvez, J., Pallav, K., Hansen, J., et al. (2014). Celiac disease or non-celiac gluten sensitivity? An approach to clinical differential diagnosis. *The American Journal of Gastroenterology*, 109(5), 741–746.
- Kaukinen, K., Turjanmaa, K., Mäki, M., Partanen, J., Venäläinen, R., Reunala, T., et al. (2000). Intolerance to cereals is not specific for coeliac disease. *Scandinavian Journal of Gastroenterology*, 35(9), 942–946.
- Kulai, T., & Rashid, M. (2014). Assessment of nutritional adequacy of packaged gluten-free food products. *Canadian Journal of Dietetic Practice and Research: A Publication of Dietitians of Canada*, 75(4), 186–190.
- Lebwohl, B., Granath, F., Ekblom, A., Smedby, K. E., Murray, J. A., Neugut, A. I., et al. (2013). Mucosal healing and risk for lymphoproliferative malignancy in celiac disease: A population-based cohort study. *Annals of Internal Medicine*, 159(3), 169–175.
- Leffler, D. (2010). Gluten intolerance: you mean I don't have celiac disease? In M. Dennis, & D. Leffler (Eds.), *Real life with celiac Disease: Troubleshooting and thriving gluten free*. (Chapter 8). Bethesda, MD: AGA Press.
- Lillie, E. O., Patay, B., Diamant, J., Issell, B., Topol, E. J., & Schork, N. J. (2011). The n-of-1 clinical trial: The ultimate strategy for individualizing medicine? *Personalized Medicine*, 8(2), 161–173.
- Ludvigsson, J. F., Reichenberg, A., Hultman, C. M., & Murray, J. A. (2013). A nationwide study of the association between celiac disease and the risk of autistic spectrum disorders. *Journal of the American Medical Association Psychiatry*, 70(11), 1224–1230.
- Makharia, A., Catassi, C., & Makharia, G. K. (2015). The overlap between irritable bowel syndrome and non-celiac gluten sensitivity: A clinical dilemma. *Nutrients*, 7(12), 10417–10426.
- Marcason, W. (2009). What is the current status of research concerning use of a gluten-free, casein-free diet for children diagnosed with autism? *Journal of the American Dietetic Association*, 109(3), 572.
- Matthias, T., Neidhöfer, S., Pfeiffer, S., Prager, K., Reuter, S., & Gershwin, M. E. (2011). Novel trends in celiac disease. *Cellular & Molecular Immunology*, 8(2), 121–125.
- Miller, S. I., Ernst, R. K., & Bader, M. W. (2005). LPS, TLR4 and infectious disease diversity. *Nature Reviews. Microbiology*, 3(1), 36–46.
- Mohamed, B. M., Feighery, C., Kelly, J., Coates, C., O'Shea, U., Barnes, L., et al. (2006). Increased protein expression of matrix metalloproteinases -1, -3, and -9 and TIMP-1 in patients with gluten-sensitive enteropathy. *Digestive Diseases and Sciences*, 51(10), 1862–1868.
- Molina-Infante, J., & Carroccio, A. (2017). Suspected nonceliac gluten sensitivity confirmed in few patients after gluten challenge in double-blind, placebo-controlled trials. *Clinical Gastroenterology and Hepatology: The Official Clinical Practice Journal of The American Gastroenterological Association*, 15(3), 339–348.
- Mooney, P. D., Aziz, I., & Sanders, D. S. (2013). Non-celiac gluten sensitivity: Clinical relevance and recommendations for future research. *Neurogastroenterology & Motility*, 25(11), 864–871.
- Moore, M. M., Schober, T. J., Dockery, P., & Arendt, E. K. (2004). Textural comparisons of gluten-free and wheat-based doughs, batters, and breads. *Cereal Chemistry*, 81(5), 567–575.
- Morant, A. (2011). *Psychosis and silent celiac disease in a down syndrome adolescent: A case report*. Case Reports in Pediatrics, 2011, 970143.
- Mowszet, K., Matusiewicz, K., & Iwańczak, B. (2014). Value of the atopy patch test in the diagnosis of food allergy in children with gastrointestinal symptoms. *Advances in Clinical and Experimental Medicine: Official Organ Wrocław Medical University*, 23(3), 403–409.
- National Institute of Allergy and Infectious Diseases. (2010). *Food allergy*. <http://www.niaid.nih.gov/topics/foodAllergy/understanding/Pages/allergicRxn.aspx> Accessed 19.05.2014.
- National Institute of Diabetes and Digestive and Kidney Diseases. (2008). *Fact sheet about celiac disease*. Bethesda, MD: National Institutes of Health. NIH Publication No. 08e4269 <http://digestive.niddk.nih.gov/ddiseases/pubs/ceciac> Accessed 19.05.2014.
- Not, T., Ziberna, F., Vatta, S., Quaglia, S., Martellosi, S., Villanacci, V., et al. (2011). Cryptic genetic gluten intolerance revealed by intestinal antitransglutaminase antibodies and response to gluten-free diet. *Gut*, 60(11), 1487–1493.
- Öhman, L., & Simrén, M. (2013). Intestinal microbiota and its role in irritable bowel syndrome (IBS). *Current Gastroenterology Reports*, 15(5), 323.
- Olén, O., Asklund, J., Ludvigsson, J. F., Hildebrand, H., Ekblom, A., & Smedby, K. E. (2011). Coeliac disease characteristics, compliance to a gluten free diet and risk of lymphoma by subtype. *Digestive and Liver Disease*, 43(11), 862–868.
- Pellegrini, N., & Agostoni, C. (2015). Nutritional aspects of gluten-free products. *Journal of the Science of Food and Agriculture*, 95(12), 2380–2385.
- Picarella, A., Di Tola, M., Marino, M., Libanori, V., Borghini, R., Salvi, E., et al. (2013). Usefulness of the organ culture system when villous height/crypt depth ratio, intraepithelial lymphocyte count, or serum antibody tests are not diagnostic for celiac disease. *Translational Research: The Journal of Laboratory and Clinical Medicine*, 161(3), 172–180.
- Pietzak, M. (2012). Celiac disease, wheat allergy, and gluten sensitivity: When gluten free is not a fad. *JPEN. Journal of Parenteral and Enteral Nutrition*, 36(1 Suppl), 68S–75S.

- Plugis, N. M., & Khosla, C. (2015). Therapeutic approaches for celiac disease. *Best Practice & Research Clinical Gastroenterology*, 29(3), 503–521.
- Roncoroni, L., Elli, L., Bardella, M. T., Perrucci, G., Ciulla, M., Lombardo, V., et al. (2013). Extracellular matrix proteins and displacement of cultured fibroblasts from duodenal biopsies in celiac patients and controls. *Journal of Translational Medicine*, 11, 91.
- Rosinach, M., Fernández-Bañares, F., Carrasco, A., Ibarra, M., Temiño, R., Salas, A., et al. (2016). Double-blind randomized clinical trial: Gluten versus placebo rechallenge in patients with lymphocytic enteritis and suspected celiac disease. *Public Library of Science*, 11(7). e0157879.
- Rostami, K., Aldulaimi, D., Holmes, G., Johnson, M. W., Robert, M., Srivastava, A., et al. (2015). Microscopic enteritis: Bucharest consensus. *World Journal of Gastroenterology*, 21(9), 2593–2604.
- Sander, G. R., Cummins, A. G., Henshall, T., & Powell, B. C. (2005). Rapid disruption of intestinal barrier function by gliadin involves altered expression of apical junctional proteins. *FEBS Letters*, 579(21), 4851–4855.
- Sanz, Y., De Pama, G., & Laparra, M. (2011). Unraveling the ties between celiac disease and intestinal microbiota. *International Reviews of Immunology*, 30(4), 207–218.
- Sapone, A., Bai, J. C., Ciacci, C., Dolinsek, J., Green, P. H., Hadjivassiliou, M., et al. (2012). Spectrum of gluten-related disorders: Consensus on new nomenclature and classification. *BMC Medicine*, 10, 13.
- Sapone, A., Lammers, K. M., Casolaro, V., Cammarota, M., Giuliano, M. T., De Rosa, M., et al. (2011). Divergence of gut permeability and mucosal immune gene expression in two gluten-associated conditions: Celiac disease and gluten sensitivity. *BMC Medicine*, 9(23).
- Sapone, A., Lammers, K. M., Mazzarella, G., Mikhailenko, I., Carteni, M., Casolaro, V., et al. (2010). Differential mucosal IL-17 expression in two gliadin-induced disorders: Gluten sensitivity and the autoimmune enteropathy celiac disease. *International Archives of Allergy and Immunology*, 152(1), 75–80.
- Schuppan, D., Pickert, G., Ashfaq-Khan, M., & Zavallos, V. (2015). Non-celiac wheat sensitivity: Differential diagnosis, triggers and implications. *Best Practice & Research Clinical Gastroenterology*, 29(3), 469–476.
- Sestak, K., & Fortgang, I. (2013). Celiac and non-celiac forms of gluten sensitivity: Shifting paradigms of an old disease. *British Microbiology Research Journal*, 3(4), 585–589.
- Shah, E., & Pimentel, M. (2014). Placebo effect in clinical trial design for irritable bowel syndrome. *Journal of Neurogastroenterology and Motility*, 20(2), 163–170.
- Shan, L., Molberg, Ø., Parrot, I., Hausch, F., Filiz, F., Gray, G. M., et al. (2002). Structural basis for gluten intolerance in celiac sprue. *Science*, 297(5590), 2275–2279.
- Simrén, M., Barbara, G., Flint, H. J., Spiegel, B. M. R., Spiller, R. C., Vanner, S., et al. (2013). Intestinal microbiota in functional bowel disorders: A Rome foundation report. *Gut*, 62, 159–176.
- Simsek, S., Baysoy, G., Gencoglan, S., & Uluca, U. (2015). Effects of gluten-free diet on quality of life and depression in children with celiac disease. *Journal of Pediatric Gastroenterology and Nutrition*, 61(3), 303–306.
- Sjöberg, V., Sandström, O., Hedberg, M., Hammarström, S., Hernell, O., & Hammarström, M. L. (2013). Intestinal T-cell responses in celiac disease – impact of celiac disease associated bacteria. *PLoS One*, 8(1). e53414.
- Sollid, L. M. (2002). Coeliac disease: Dissecting a complex inflammatory disorder. *Nature reviews. Immunology*, 2(9), 647–655.
- Sollid, L. M., & Jabri, B. (2011). Celiac disease and transglutaminase 2: A model for posttranslational modification of antigens and HLA association in the pathogenesis of autoimmune disorders. *Current Opinion in Immunology*, 23(6), 732–738.
- Theethira, T. G., & Dennis, M. (2015). Celiac disease and the gluten-free diet: Consequences and recommendations for improvement. *Digestive Diseases*, 33(2), 175–182.
- Thompson, T., & Simpson, S. (2015). A comparison of gluten levels in labeled gluten-free and certified gluten-free foods sold in the United States. *European Journal of Clinical Nutrition*, 69(2), 143–146.
- Tortora, R., Capone, P., De Stefano, G., Imperatore, N., Gerbino, N., Donetto, S., et al. (2015). Metabolic syndrome in patients with coeliac disease on a gluten-free diet. *Alimentary Pharmacology & Therapeutics*, 41(4), 352–359.
- Uhde, M., Ajamian, M., Caio, G., De Giorgio, R., Indart, A., Green, P. H., et al. (2016). Intestinal cell damage and systemic immune activation in individuals reporting sensitivity to wheat in the absence of coeliac disease. *Gut*, 0, 1–8.
- Verdu, E. F., Huang, X., Natividad, J., Lu, J., Blennerhassett, P. A., David, C. S., et al. (2008). Gliadin-dependent neuromuscular and epithelial secretory responses in gluten-sensitive HLA-DQ8 transgenic mice. *American Journal of Physiology. Gastrointestinal and Liver Physiology*, 294(1), G217–G225.
- Visser, J., Rozing, J., Sapone, A., Lammers, K., & Fasano, A. (2009). Tight junctions, intestinal permeability, and autoimmunity: Celiac disease and type 1 diabetes paradigms. *Annals of the New York Academy of Sciences*, 1165, 195–205.
- Vogelsang, H., Schwarzenhofer, M., Steiner, B., Wyatt, J., & Oberhuber, G. (2001). In vivo and in vitro permeability in coeliac disease. *Alimentary Pharmacology & Therapeutics*, 15(9), 1417–1425.
- Volta, U., Caio, G., Tovoli, F., & De Giorgio, R. (2013). Non-celiac gluten sensitivity: Questions still to be answered despite increasing awareness. *Cellular & Molecular Immunology*, 10(5), 383–392.
- Volta, U., & De Giorgio, R. (2012). New understanding of gluten sensitivity. *Nature Reviews. Gastroenterology & Hepatology*, 9(5), 295–299.
- Volta, U., Tovoli, F., Cicola, R., Parisi, C., Fabbri, A., Piscaglia, M., et al. (2012). Serological tests in gluten sensitivity (non celiac gluten intolerance). *Journal of Clinical Gastroenterology*, 46(8), 680–685.
- Volta, U., & Villanacci, V. (2011). Celiac disease: Diagnostic criteria in progress. *Cellular & Molecular Immunology*, 8(2), 96–102.
- Vriezinga, S. L., Auricchio, R., Bravi, E., Castillejo, G., Chmielewska, A., Crespo Escobar, P., et al. (2014). Randomized feeding intervention in infants at high risk for celiac disease. *The New England Journal of Medicine*, 371(14), 1304–1315.
- Wahnschaffe, U., Schulzke, J. D., Zeitz, M., & Ullrich, R. (2007). Predictors of clinical response to gluten-free diet in patients diagnosed with diarrhea-predominant irritable bowel syndrome. *Clinical Gastroenterology and Hepatology*, 5, 844–850.
- Wahnschaffe, U., Ullrich, R., Riecken, E. O., & Schulzke, J. D. (2001). Celiac disease-like abnormalities in a subgroup of patients with irritable bowel syndrome. *Gastroenterology*, 121(6), 1329–1338.