Comparisons of Celiac Disease and Non-Celiac Gluten Sensitivity

Victoria Thomas

A Senior Thesis submitted in partial fulfillment
of the requirements for graduation
in the Honors Program
Liberty University
Spring 2020

Acceptance of Senior Honors Thesis

This Senior Honors Thesis is accepted in partial
fulfillment of the requirements for graduation from the
Honors Program of Liberty University.

_____
Kimberly Mitchell, Ph.D.
Thesis Chair

_____
Greg Raner, Ph.D.
Committee Member

_____
Cindy Goodrich, Ed.D.
Assistant Honors Director

_____
Date

Abstract

Celiac disease (CD) and non-celiac gluten sensitivity (NCGS) are often confused or grouped together due to their commonalities. However, this is careless behavior because there are clinically significant differences between the two diseases. Similarities between them include varying degrees of damage or permeability in the lining of the small intestine, involvement of the innate immune system, alleviation of symptoms upon implementation of a gluten-free diet (GFD), and the possibility for complications if the pathology is not adequately treated. Despite these similarities, minor details such as the following make CD and NCGS worth differentiating: the question of gluten as the true trigger for NCGS, severity of villous atrophy present in only CD, psychiatric comorbidities present in NCGS, and possibility of a less restrictive treatment for NCGS using gluten detoxification.

Comparisons of Celiac Disease and Non-Celiac Gluten Sensitivity

Introduction

Americans are becoming increasingly aware of what they are consuming through their diets. Recently, a large number of people have been removing gluten from their diet in an attempt to alleviate a wide variety of intolerance or allergy-like symptoms (Reilly, 2016). However, not all of these consumers are making educated choices when it comes to removing dietary gluten. One study found that many people who were opting for gluten-free alternative foods were buying these products because they thought it would be healthier, improve overall digestive health, aid in weight loss, or, least likely, they had a gluten sensitivity (Gaesser & Siddhartha, 2012; Reilly, 2016). However, this careless adoption of the gluten free diet (GFD) is irresponsible due to the nutritional deficiencies that can accompany the diet. Gluten has been condemned as evil by many who are not educated in the characteristics of the molecule. Gluten itself is not unhealthy, toxic, or bad for those who do not have Celiac disease (CD) or non-celiac gluten sensitivity (NCGS) (Reilly, 2016). Gluten is a general name for a class of alcohol-soluble proteins present in wheat, barley, and rye (Biesiekierski, 2017). This class of proteins contains gliadin and glutenin, which are both characterized by high levels of glutamine and proline amino acids. Although this renders the protein difficult to digest, adverse immune reactions because of this are only observed in patients with CD and NCGS (Biesiekierski, 2017).

CD and NCGS are similar gluten-related gastrointestinal diseases that are increasing in prevalence at a rapid pace (Green et al., 2015). A distinction between these two diseases is vital to precise, accurate care of the patient. Many patients suffer through symptoms for months to

years before an accurate diagnosis and subsequent treatment plan is made. CD is an autoimmune disease characterized by damage to the epithelial lining of the small intestine prompted by the ingestion of gluten-containing foods (Elli et al., 2015). NCGS is currently a disease of exclusion or last resort after thorough testing has been conducted for CD. However, both pathologies are characterized by a gluten trigger, compromised intestinal epithelia, gluten-free diet treatment, and potential for comorbidities (Elli et al., 2015). Most reports suggest the prevalence of CD in America to be low but steadily increasing (Green et al., 2015). Similar data suggests that the incidence rate of NCGS is higher than that of CD, but this data is unreliable due to the lack of distinction between NCGS and CD, lack of specific biomarkers for NCGS, and self-diagnosis of NCGS (Fasano & Catassi, 2012). Both CD and NCGS affect the function of the small intestine by damaging the epithelial cells that line the lumen, thereby hindering absorption and secretion of nutrients. The exact mechanism for how this occurs in NCGS is unknown, but CD pathogenesis and pathophysiology is well documented (Leonard et al., 2017). Since NCGS is largely a diagnosis of exclusion, diagnostic testing for both CD and NCGS relies on traditional methods for diagnosing CD such as serology, histology, and genetic testing. NCGS is clinically defined as an alleviation of symptoms after removal of gluten from the diet. These patients have negative celiac serology and duodenal histology, while CD patients have positive serology, histology, and genetic predisposition (Kabbani et al., 2014; Collyer & Kaplan, 2016). Since NCGS is clinically defined as the alleviation of symptoms after removing gluten from the diet, the most effective treatment is a gluten-free diet (Allen, 2016). This is also the only accepted treatment for CD, but celiac patients require much stricter adherence due to the severity and nature of their symptoms. Even inadvertent consumption of minor amounts of gluten such as that

from cross-contamination with gluten-containing foods can lead to intestinal damage in those with CD (Dieterich & Zopf, 2019). Current treatment for both of these diseases consists of mere avoidance of symptoms rather than altering pathophysiology or pathogenesis (Allen, 2016). Further research of the mechanisms and triggers of both CD and NCGS is necessary for more specific, effective treatment to be developed. This is vital to the health of these patients because misdiagnosis, lack of adherence to treatment, and lack of treatment altogether may lead to the development of comorbidities and complications such as increased risk of mortality, osteoporosis, malnutrition, reproductive complications, and development of other autoimmune disorders (Coqueiro et al., 2017). As CD and NCGS increase in prevalence and consumers become more aware of their diets, a clear distinction between these two diseases must be made in an effort to provide more specific, effective treatment for each population of patients.

Epidemiology

Accurate prevalence data for CD and NCGS are difficult to obtain due to the complex nature of each disease, lack of biomarkers for NCGS, and overlap of symptomology between the two diseases resulting in misdiagnoses (Green et al., 2015). The clinical recognition of NCGS is relatively new, so more epidemiological studies must be conducted in order to accurately describe the prevalence of the disease. It has been hypothesized, however, that the prevalence of this disease is higher than that of CD (Fasano & Catassi, 2012). Roughly 1% of the American population is estimated to have clinically diagnosed CD (Green et al., 2015). However, other countries such as Finland and England report higher incidence rates. This may be due to a discrepancy in healthcare accessibility. Increasing prevalence in developing countries may be due to globalization in regard to the wheat-filled western diet. These countries are consuming

more wheat, so more of the population will begin to express wheat or gluten related sensitivities. Another reason this data should be interpreted with critical thinking is that the CD diagnosis is often missed. Clinically, it is common for children to present with frequent infection, malnutrition, and diarrhea but not be tested for CD. It is also of importance to note that CD is diagnosed in women three times as often as in men. The reason for this remains unclear but may be due to higher rates of autoimmune diseases in general in women and a higher likelihood that a female would interact with a healthcare provider as a result of concerning health symptoms (Green et al., 2015).

CD was first recognized in the late 1940s when gluten was identified as a trigger for an unidentified set of symptoms characterized by malnutrition. Decades later, researchers and clinicians gained more knowledge as they studied the immune response that takes place in these patients following the ingestion of gluten. This led to the discovery of the autoimmune nature of the disease (Murray et al., 2018). As this disease is further studied and understood, the number of diagnoses also increases (Catassi et al., 2010). One study found the prevalence of CD among adult American men to be 0.2% in the 1950s and 1.0% in the early 2000s. This trend was also seen in other countries such as Finland, where the incidence rate climbed from 1.05% to 1.99%. The incidence rate in the US alone has rapidly increased, doubling almost every 15 years (Catassi et al., 2010). NCGS was first described in the early 1980s when clinicians described a group of patients who appeared to be gluten-sensitive without exhibiting signs of CD (Leccioli et al., 2017). However, it was not truly associated with CD and wheat allergy until 2012 due to nomenclature and clinical parameter debates (Leccioli et al., 2017).

Despite once being thought to have only occurred in Caucasian populations, similar incidence trends for CD have been observed in North Africa, India, and Middle Eastern countries (Catassi et al., 2015). Saharawi Africans demonstrate the highest known prevalence rates of CD at 5.6%. The reason for this is unknown. Asian populations tend to have the lowest prevalence rates, likely due to lower dietary consumption of wheat, which reduces gluten intake, and a low frequency of the allele associated with genetic predisposition to CD (Catassi et al., 2015). Although developing countries have similar rates of incidence as the United States and European countries, rates of diagnosis are far lower due to poor disease awareness and low accessibility to healthcare (Catassi et al., 2010). The incidence rate of developing countries is likely to continue to increase as western dietary trends, such as increased consumption of wheat, are adopted. The reason for increasing incidence in America is unclear but may be at least in part due to increased consumption of gluten products such as wheat, barley, and rye, mutational changes to the gluten protein itself, and bacterial changes in the gut (Catassi et al., 2010). Yet another reason may be the development of better diagnostic tools such as genetic testing and more specific, targeted serological testing (Murray et al., 2018). For these reasons, prevalence of NCGS is also expected to increase. Further epidemiological studies of NCGS are necessary to obtain accurate incidence rates in America and around the world.

Pathophysiology

Both CD and NCGS affect the function of the small intestine by damaging the epithelial cells that line the lumen, thereby hindering absorption and secretion of nutrients (Leonard et al., 2017). The exact mechanism for how this occurs in NCGS is unknown, but CD pathogenesis and pathophysiology is well documented. It is known, however, that alpha amylase/trypsin inhibitors

may be a trigger for NCGS along with or instead of gluten. In addition to this, NCGS patients also have reduced levels of T-regulatory cells and elevated levels of intraepithelial lymphocytes in the intestine (Leonard et al., 2017). The implication of this will be discussed further following the discussion of normal gastrointestinal (GI) physiology and CD pathophysiology.

GI physiology involves the absorption and digestion of nutrients such as water, vitamins, and electrolytes from food. However, the GI system also has another important role in the body. It acts as a protective barrier between the inside of the body and the outside world (McLaughlin, 2009). The GI system breaks down food in a variety of ways, but a primary mechanism is via enzymes. These gastric, pancreatic, and epithelial enzymes interact with food in the stomach and small intestine. Cephalic secretion of digestive enzymes occurs when food enters the mouth or is sensed outside of the body and the brain prepares the body for digestion. Gastric secretion occurs when food reaches the stomach and the partially digested food triggers the release of gastrin. Following this, food enters the intestines and triggers the release of acid, gastrin-28, and other peptidases. Gastrin-28 stimulates histamine secretion, which in turn stimulates further acid secretion by activating H^+-K^+ ATPase (McLaughlin, 2009). At the tissue level, normal physiology of the lower GI tract consists of four layers of tissue with respect to the lumen from superficial to deep: mucosa, submucosa, muscularis, and serosa (Bischoff et al., 2014). The functional barrier between the lumen of the intestine and the submucosal layers consists of a thin layer of epithelial cells connected paracellularly through tight junctions. These tight junctions allow water, ions, and small molecules to flow through the epithelial lining, both to and from the intestinal lumen. The junctions also function to prevent antigens from entering the mucosal layers through the implementation of occludin and claudin proteins linking adjacent

cytoskeletons of cells. Since claudin proteins are transcellular, integrity of the intestinal epithelium is largely determined by these proteins (Bischoff et al., 2014). Epithelial tissue consists of a villous, highly differentiated edge and a basal intestinal crypt in which stem cells divide and mature (Yen & Wright, 2006). Stem cells divide, mature, and undergo apoptosis in a rapid cycle in order to replenish the functional, active epithelial tissue. Differentiated villous cells constitute the functional characteristic of the tissue with their absorption and protective properties, while stem cells constitute the proliferative characteristic of the tissue. There are four fates for intestinal stem cells: enterocytes, goblet cells, enteroendocrine cells, and Paneth cells. Columnar cells, also called enterocytes, are responsible for absorption and secretion through tight junctions and cell membrane transporters. Goblet cells protect and maintain the surface of intestinal mucus by producing mucin. As previously mentioned, the epithelium also contains endocrine cells. Deep to these in the base layer, Paneth cells play an important role in maintaining stem cell homeostasis (Yen & Wright, 2006). The integrity of the small intestinal lining is critical to efficient functioning of the organ. When the integrity is compromised, digestion, secretion, absorption, and immune protection are also compromised (Bischoff et al., 2014).

In CD, the small intestinal lining is damaged by mechanism of cytotoxic lymphocytes in an autoimmune manner following the ingestion of gluten (Meresse et al., 2015). This damage, characterized by villous flattening and crypt widening, is catalyzed by the ingestion of dietary gluten in genetically predisposed individuals. The genetic predisposition best described is the expression of MCH class II haplotypes encoding the antigen presenting molecules HLA-DQ2.5 and HLA-DQ8. Ingestion of gluten in these individuals, as well as other factors such as

environment and gut microbiota, leads to chronic activation of intraepithelial lymphocytes such as CD4+ T cells and IgA cells in plasma. However, other factors such as overproduction of cytokines and inhibition of regulation are also necessary to induce an autoimmune response. Cytokines such as interleukin 15 (IL-15) and interleukin 17 (IL-17), and suppression of regulatory T cells, are essential innate immune system components that aid in the progression of CD (Meresse et al., 2015).

CD is often confused with an allergy to gluten. Unlike true allergies, however, CD is not an IgE mediated, immediate response to the ingested antigen. Instead, gluten is partially digested into gliadin fragments and then deamidated by tissue transglutaminase, which is upregulated in patients with CD (Kagnoff). This renders it a more immunogenic protein and binds to intraepithelial lymphocytes that express the antigen presenting molecules HLA class II DQ2.5 and DQ8 on their surface such as macrophages, dendritic cells, and B cells. These cells then present the antigen, gliadin, to specific CD4+ T cells. T helper cells then release pro-inflammatory cytokines such as interferon gamma, IL-15, and IL-17. This results in differentiation of intraepithelial lymphocytes into cytotoxic T cells and produces, alongside cytokines, inflammation of the intestinal wall and villous atrophy (Green et al., 2015). CD develops as a result of multiple things. Some of these include a genetic predisposition for certain antigen-presenting cells, but it has also been suggested that certain viral infections may also play a role in predisposing individuals to CD (Bouziat et al., 2017). Reovirus in particular has been of interest in recent research; it is thought that infection with reovirus suppresses intraepithelial lymphocyte differentiation into regulatory T cells and promotes T helper cell sensitivity to gluten. In an experiment testing a form of reovirus in mice, researchers found that reovirus

infection induced a loss of tolerance to gliadin. This was determined by the presence of anti-gliadin IgG antibodies and a delayed hypersensitivity reaction. Reovirus also activated tissue transglutaminase (TTG). In a healthy individual, TTG is normally inactive until it is transported out of the cell and activated by calcium before it is rapidly inactivated (Kupfer & Jabri, 2012). However, in an inflammatory environment or in the presence of reovirus, TTG remains active (Bouziat et al., 2017). This is significant because TTG deamidates gluten, which converts glutamine to glutamate and leaves the peptide with more negative charges than before (Kupfer & Jabri, 2012). Since HLA-DQ2 and HLA_DQ8 have positively charged areas specific for negatively charged antigens, this increased presence of negative charge on the gluten peptides increases the affinity of HLA molecules for gluten peptides (Kupfer & Jabri, 2012). In summary, reovirus increased the likelihood that gluten peptides would bind HLA molecules and be presented to T cells (Bouziat et al., 2017). Furthermore, in another part of that same study, patients were analyzed for various virus titers. Patients with CD had significantly high titers for reovirus and made up the majority of individuals with high titers in general. The exact mechanisms of how viruses such as reovirus initiate loss of tolerance to a dietary antigen and introduce T helper cell immunity against dietary antigen remain unclear. However, this study argues that reovirus, along with other enteric viruses, disrupts intestinal immune homeostasis enough to induce loss of gluten tolerance (Bouziat et al., 2017).

Research of important cytokines and immunogens involved in CD is necessary because it may lead to the creation of a more effective treatment. It also may lead to a better understanding of the mechanisms of NCGS. As mentioned previously, IL-15, IL-17, and cytotoxic T cells play a major role in the pathophysiology of CD (Meresse et al., 2015; Sjöberg, 2013; Cook et al.,

2017). IL-15 belongs to the same class of cytokines as IL-2. Both of these cytokines have the ability to stimulate CD8+ T and natural killer cell cytotoxicity in vitro. However, IL-2 is responsible for maintaining T regulatory cell homeostasis and survival in vivo, which is why knockout, or blocking, of this cytokine often results in autoimmunity development. When IL-15 is blocked or taken out of the physiological equation, malignancies can develop in natural killer cells, memory T cells, and intestinal intraepithelial lymphocytes. In CD, there is a large increase in the number of intraepithelial lymphocytes. These in turn upregulate granzyme B, which is also under regulation of IL-15 and responsible for cytotoxicity of T cells and NK cells. Since IL-15 expression is upregulated in CD, this impacts the pathogenesis of the disease in several ways. The upregulation of IL-15 may cause the accumulation of intraepithelial lymphocytes by communicating an antiapoptotic signal (Malamut et al., 2010). Another way IL-15 may play a role in the pathophysiology of CD is by organizing cytolytic activity in the small intestinal epithelium (Meresse et al., 2015). IL-15 stimulates granzyme B, which increases expression of natural killer cell receptors on CD8+ T cells. This renders the cell more cytotoxic than before. IL-15 may also impair local immunoregulation by impairing the conversion of T cells into T regulatory cells or by rendering CD8+ and CD4+ T cells less responsive to the suppressive action of the T regulatory cells (Meresse et al., 2015). IL-17 is a pro-inflammatory cytokine that is typically observed in higher levels in CD patients (Sjöberg, 2013). Cytotoxic T cells that secrete this cytokine are thought to become hyper-stimulated with lost specificity due to the impaired function of their immunoregulator, T regulatory cells. Certain rod-shaped bacteria commonly found in the gut of CD patients have been hypothesized to stimulate the production of IL-17 (Sjöberg, 2013). As mentioned before, T regulatory cells play a vital role in CD

pathophysiology (Cook et al., 2017). It has been observed that CD patients express impaired function of T regulatory cells or impaired signaling via IL-15. FOX protein 3 (FOXP3) is a transcription factor necessary for differentiation and function of T regulatory cells (Meresse et al., 2015). It has been observed that the levels of FOXP3 T regulatory cells in CD patients following gluten ingestion are abnormally high. Specifically, 80% of all circulating gluten specific CD4+ T cells consisted of FOXP3 T regulatory cells. This seems to be the body's way of attempting to regain homeostasis, but the signal is interrupted. This is one of the sources of the autoimmunity of CD (Cook et al., 2017).

NCGS is similar to CD in terms of pathophysiology, but some differences are worth noting. NCGS is an enteropathy characterized by a number of GI symptoms that are alleviated following the removal of gluten from the diet (Leccioli et al., 2017). These symptoms are likely due to the presence of enterocyte damage and increased permeability in the intestinal lining, allowing translocation of GI contents to the blood. NCGS is similar to CD in that it is not a true, immediate allergic reaction mediated by IgE. In fact, symptoms may take up to hours or days to appear after the ingestion of gluten. NCGS is a relatively new diagnostic term, so there are many gaps in research regarding this pathology. However, it is known that it utilizes the innate immune response following the ingestion of the trigger. For example, gluten has been questioned as the true trigger for NCGS. It has been hypothesized that fermentable saccharides and polyols, wheat amylase trypsin inhibitors (ATIs), or wheat germ agglutinin could also trigger the immune response observed in NCGS. Most likely, however, all of these triggers play a role in the pathogenesis and propagation of this disease (Leccioli et al., 2017). It has been shown that NCGS patients often demonstrate increased expression of toll-like receptors, which bind to

wheat ATIs and activate dendritic cells. Interestingly, it has been shown that modern wheat ATIs have a higher affinity for toll-like receptor 4 than older wheat ATIs (Cabanillas, 2019). This suggests that ATIs have a more significant role in NCGS pathogenesis than what is currently attributed to these molecules. It is worth noting a smaller hypothesis involving glyphosate as a possible factor leading to the development of NCGS (Samsel & Seneff, 2013). Glyphosate is a widely used herbicide that has been subject to questioning and debate over safety data. This compound is potentially harmful to the human body, even in small doses, in several ways. It decreases the number of beneficial bacteria in the gut and therefore allows overgrowth of harmful bacteria, breaks down vital minerals in the body, and interferes with many vital enzymes. One study demonstrated several examples of gastrointestinal damage in fish that were exposed to glyphosate. Enzymes involved in protein, fat, and starch digestion were impaired in these fish, and a biopsy also revealed damage to the mucosal lining of the intestine. This evidence is remarkably similar to tissues obtained from individuals with CD, but it cannot be applied to human subjects before human studies involving glyphosate are conducted (Samsel & Seneff, 2013). An additional argument in the glyphosate-trigger hypothesis is the relationship between the composition of gut bacteria in CD patients and the antimicrobial properties of glyphosate. CD patients often have reduced levels of beneficial bacteria *Enterococcus, Bifidobacteria, and Lactobaccilus* (Cagno et al., 2011), all of which are readily eliminated by glyphosate (Sansel & Seneff, 2013). This seems to support the hypothesis that glyphosate triggers the development of NCGS, but that conclusion cannot be made based solely on correlational data. In contrast to the argument that glyphosate could lead to development of diseases such as NCGS, some studies argue that the relationship between NCGS disease

pathogenesis and glyphosate exposure is only correlational (Mesnage & Antoniou, 2017). Although glyphosate use and prevalence of gastrointestinal diseases such as NCGS have both increased in recent years, it is not acceptable to attribute this as causational data. No studies have been conducted in such a manner that could lead to a causational conclusion (Mesnage & Antoniou, 2017). However, since there is correlational data between increased use of glyphosate and prevalence of NCGS, in addition to significant data showing glyphosate can cause certain cancers, this compound needs to be studied further as a potential environmental trigger for the pathogenesis of gastrointestinal diseases such as NCGS (Samsel & Seneff, 2013). Overall, all that is known about NCGS pathophysiology is that an adaptive immune response occurs, increased levels of intraepithelial lymphocytes are present, and increased intestinal permeability seems to be present (Lebwohl et al., 2015). Further research of the physiological mechanism of NCGS is vital to understanding the disease, coming up with clinical biomarkers, and developing a more specific treatment.

Diagnosis

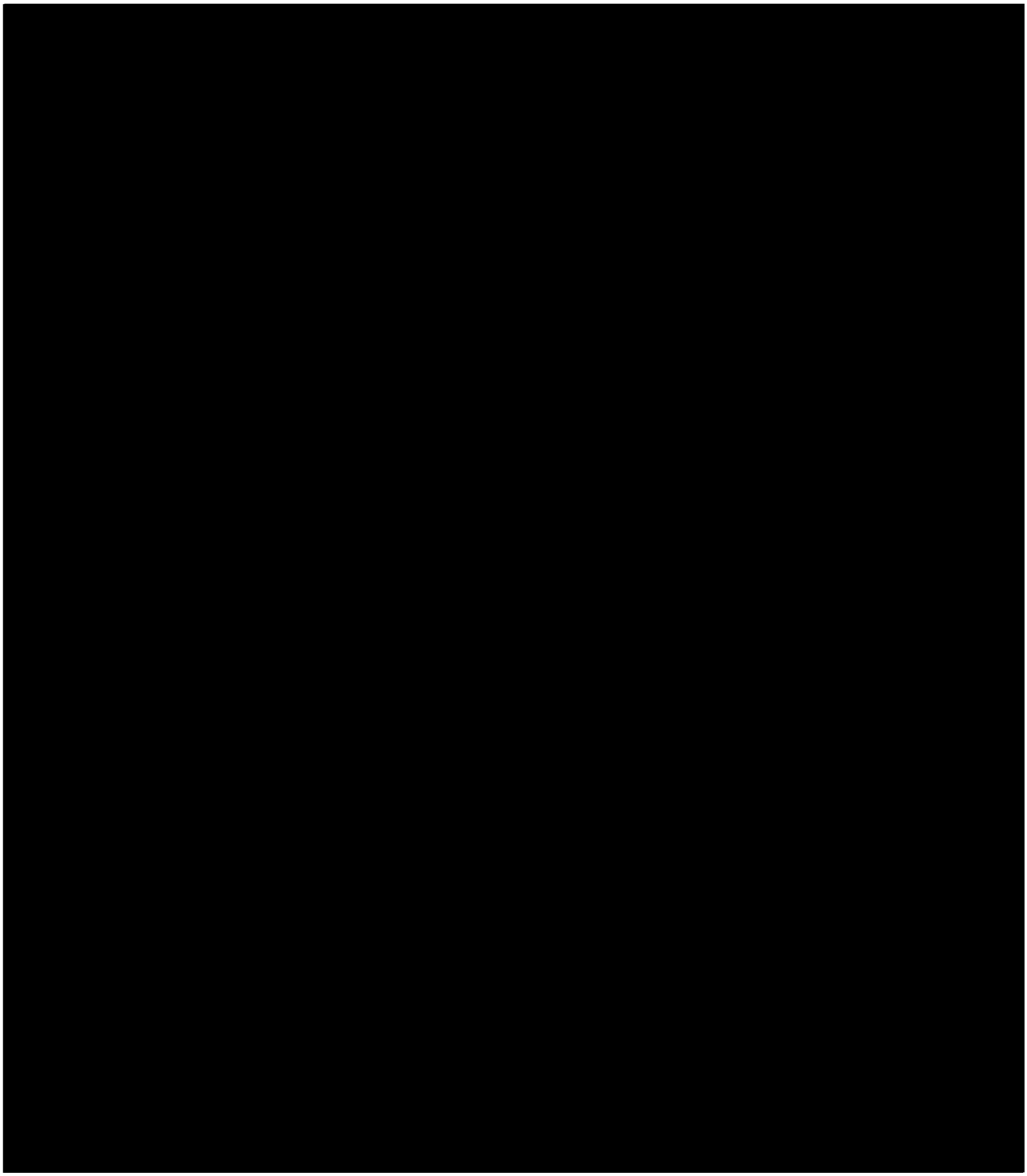
The range of symptoms for CD and NCGS is vast, so there is frequent overlap between the two diseases (Elli et al., 2015). This not only makes them difficult to pinpoint in a clinical setting but also makes it difficult to discern between the two. However, NCGS often presents with a wider range of extra-intestinal symptoms such as headaches, malaise, and anemia. Currently, NCGS is strictly diagnosed after thorough testing for other gluten-related disorders has been conducted and come back as negative (Elli et al., 2015). As for CD, general risk is first considered by taking a patient family history of CD or other autoimmune disorders (Allen, 2016). If adequate risk is determined, then serological screening of the intestinal mucosa is

conducted. This uses enzyme-linked immunosorbent assay (ELISA) to evaluate IgA antibodies to tissue transglutaminase or deamidated antigliadin antibodies. This generally gives a 91-99% accuracy in identifying CD. However, it is not as accurate or esteemed as highly as biopsy of the proximal small intestine, otherwise known as the duodenum. This is shown in relation to the rest of the GI tract in Figure 1. This lack of complete accuracy is why patients with positive serological markers are often further tested by biopsy to evaluate the levels of lymphocytes and atrophy of the villi in the epithelial lining of the proximal small intestine. If the patient has positive serology and positive biopsy, then a CD diagnosis is confirmed. If serology is positive and biopsy is negative, then a diagnosis is deferred and a biopsy is conducted again at a later date (Allen, 2016). Kabbani et al. (2014) demonstrated a specific method for clinical discernment between CD and NCGS. In this study, genetic testing was incorporated early in the diagnostic process rather than as a final step, which is common practice. The absence of HLA genes gives a clearly negative CD diagnosis and leads to the indication of NCGS. This would limit the number of endoscopies performed to obtain a definitive diagnosis (Kabbani et al., 2014). More research needs to be conducted to obtain a better understanding of the underlying mechanisms of NCGS; this will lead to a more specific, efficient method for diagnosing the pathology against CD than the current method of exclusion.

Treatment

Since both CD and NCGS are worsened by the ingestion of gluten, the best form of treatment is to avoid the ingestion of gluten (Allen, 2016). Due to the autoimmune nature and severity of CD, these patients are forced to adhere to a life-long, strict gluten-free diet (GFD). NCGS is also treated with a GFD, but in some cases the immune response can be challenged by

FIGURE



reintroducing gluten into the diet after some time. Since much is still unknown about NCGS, there are many possible forms of treatment that have the potential to be effective. One such treatment is based on the theory regarding fermentable saccharides and polyols (FSAP) as the true trigger of symptoms rather than gluten (Dieterich et al., 2019). A low FSAP diet has been shown to improve symptoms associated with NCGS. However, this could be due to the overlap of foods in the low FSAP diet and the GFD. Eliminating fermentable saccharides usually involves eliminating wheat, which also eliminates gluten (Dietrich et al., 2019). This is also the case with ATIs. A GFD usually eliminates foods containing ATIs and FSAPs and vice versa (Cabanillas, 2019). Future studies in this area could incorporate forms of treatment commonly used to treat IgE mediated allergies such as immunotherapy. Allergen immunotherapy (AIT) delivers small doses of the allergen, increasing in dosage over time, with the aim to desensitize the immune system to the antigen (Hoffman et al., 2017). Types of AIT currently being studied, not specifically for NCGS, include sublingual, oral, subcutaneous, intradermal, and epicutaneous immunotherapies (Wood, 2016; Yasuda, Ura, Taniguchi, & Yoshida, 2016). Within these various forms of AIT, peptide immunotherapy and the use of recombinant allergens have the potential to make these strategies more effective (Hoffman et al., 2017). AIT is a promising treatment for individuals suffering from IgE mediated, true allergies and therefore may be effective for creating tolerance in NCGS patients. Further study of effectiveness of specific types of AIT in treatment of NCGS is needed.

Research of novel CD treatments is highly encouraged for many reasons, one being the differentiation of treatment for CD versus NCGS. The current GFD treatment eliminates the trigger for the inflammation and atrophy of villi in the intestinal epithelial lining (Chander et al.,

2018). Strict adherence to the GFD allows time for the lining of the intestine to heal, which usually occurs within 6 to 24 months of the diet onset. Following this diet according to Codex Alimentarius guidelines for safe gluten consumption allows for a maximum of 20 ppm of gluten in "gluten-free" products. The Food and Drug Administration has yet to define an acceptable, safe gluten threshold for those with CD, which demands more attention be given to this increasingly prevalent disease (Fasano & Catassi, 2012). In addition, patient compliance and adherence to the GFD is often low due to the social and economic challenges that accompany implementation of the diet (Capacci et al., 2018). Gluten-free foods and gluten-alternative foods are notoriously expensive and relatively limited. Although gluten-free alternatives to food have been steadily increasing in availability within recent years, there is still a lack of affordable options. In a survey of a sample of grocery stores in the United States, common GF alternatives were found to be 2-4 times more expensive than the gluten-containing counterparts (Capacci et al., 2018). This, combined with the lack of availability, is a severe hinderance to the patient's adherence to a GFD. Expense and availability are also impacted by the new wave of individuals self-diagnosing themselves as gluten intolerant and adopting a GFD either as a possible treatment or simply as a seemingly healthier lifestyle. This drives the price of gluten-free alternatives higher for those patients who are on this diet as a necessity. Educating consumers and patients in the differences between gluten-related disorders that do not necessitate a GFD and those that do, such as CD, will alleviate a multitude of problems and will benefit CD and NCGS patients. In addition to the logistical challenges of a GFD, social implications exist as well. Social pressure makes it difficult to follow a strict diet and eat the same way an individual's peers eat in order to avoid feeling left out or unaccepted. This may lead to less compliance to the

diet in an attempt to avoid being ostracized from one's social group. Especially in America, where gluten-containing products make up a significant part of the diet, new CD patients may experience social recoil when they can no longer eat what all of their peers are eating (Capacci et al., 2018).

Since there are considerable hinderances in the way of strict adherence to a GFD, new forms of treatment must be studied. Novel forms of treatment for CD include therapeutic strategies such as alternative cereals or modified wheat, gluten-detoxifying, inhibiting gliadin transport across the intestinal epithelium, and immune modulation (Discepolo & Guandalini, 2017). Gluten detoxification involves the interaction of gluten peptides with digestives such as probiotics, oral protease supplements, and essentially encasing the molecule in a polymer so that it does not have the opportunity to come into contact with the lymphocytes in the intestinal epithelium. Inhibiting transcellular movement of gliadin across the intestinal lining involves the use of tight junction modulators to decrease overall permeability. This can be achieved using drugs such as larazotide acetate. Drugs can also be used to administer immune modulatory strategies such as anti-IL-15 antibodies, deamidation blocking, and to inhibit presentation of gliadin on antigen presenting cells. Due to clinical research costs, safety, and efficacy concerns, the detoxification of gluten appears to be the most feasible, effective alternative CD treatment (Discepolo & Guandalini, 2017). AGY is an antibody derived from chicken egg yolk that has demonstrated the potential to neutralize gliadin (Sample et al., 2017). This polyclonal antibody is ideal for the neutralization of gluten proteins because it is cost effective, simple to isolate, and is not absorbed across the intestinal epithelium into the blood. A study by Sample et al. was conducted to evaluate the efficacy of this Food and Drug Administration approved supplemental

antibody in addition to a GFD for CD patients looking to minimize accidental exposure to gluten, which is all too common in a western diet. Although the study participants were already on a GFD, a general trend of symptom alleviation was observed following incorporation of AGY. In addition, the normal levels of anti-tissue transglutaminase and anti-gliadin IgA and IgG decreased over the trial period (Sample et al., 2017). Further research of AGY with larger sample populations is necessary to thoroughly evaluate the efficacy of AGY as a treatment for CD (Discepolo & Guandalini, 2017). However, this antibody does demonstrate promise, at least as a supplemental treatment to the GFD. AGY supplementation may allow patients with CD to occasionally deviate from a GFD in small proportions with minimal increase in symptoms. The efficacy of this treatment for NCGS should also be an area of future research, as these patients may experience symptoms on a dosage dependent basis and would then benefit from neutralizing any percentage of their gluten intake (Discepolo & Guandalini, 2017).

Complications

When CD is left untreated or treated ineffectively with poor adherence to treatment, complications and malignancies often arise (Malamut & Cellier, 2015). Loss of bone density is a common complication of CD, as calcium is not appropriately absorbed through the intestinal lining. This is present in 50-70% of all CD patients, but at least partial correction is possible under treatment with a GFD. Treatment for this complication includes calcium and vitamin D supplementation. Other autoimmune disorders are closely related to CD. Some CD patients will develop autoimmune complications, most commonly T1D or autoimmune thyroiditis. Another complication of CD is T cell lymphoma. The incidence rate for this complication is 0.024 per 100,000 people in the US. Lymphoma is most common in CD patients whose symptoms were

non-responsive to a GFD, referred to as having refractory CD. Other complications that can arise from untreated CD include impaired spleen function, neuropathic disorders, infertility, and ulcerative jejunoileitis (Fasano & Catassi, 2012). Hyposplenism is present in over 33% of CD patients, but it is easily reversed with implementation of a GFD. Low spleen function in celiac patients often results in low iron levels in the blood, which may or may not accompany anemia (Di Sabatino et al., 2013). CD associated neuropathy such as nausea, motor ataxia, loss of balance, or general muscle weakness is only sometimes associated with CD. Approximately 10% of CD patients experience neuropathological symptoms. Data concerning the relationship between CD and neuropathy is limited, but it is clear that there is a correlation between the two. Some patients report neurological symptoms before the diagnosis of CD, while others report the development of symptoms after the diagnosis of CD. This could simply be due to late versus early diagnosis. Data on the effect of a GFD on various forms of neuropathy in CD patients remains inconclusive and warrants further study (Rigamonti et al., 2007). The relationship between infertility in women and CD has also been somewhat inconclusive. Most recent studies, however, have shown that CD only leads to infertility in untreated cases. Normal reproductive function is often restored after implementation of a GFD (Zugna et al., 2010). Other risks such as cardiovascular disease and nutritional deficiency are also common (Bathrellou et al., 2018). Gravely, the mortality rate of CD patients has been estimated as high as 39% prior to diagnosis and initiation of treatment. This drops significantly each year the patient continues with the GFD. Lymphoproliferative malignancies are an especially common morbidity in patients with CD, more often found in those with refractory CD. Yet another complication observed in CD patients is gallbladder disease (Rubio-Tapia & Murray, 2007). Due to damage in the mucosal lining of

the intestine, some CD patients do not release enough cholecystokinin after food ingestion. This prevents the gallbladder from emptying and therefore renders it more susceptible to the formation of stones or blockages. However, normal function of the gallbladder often returns after implementation of a GFD (Rubio-Tapia & Murray, 2007). Although the risk factor for these malignancies is relatively high, the incidence of these malignancies in CD populations is low. Most of these risk factors and complications are reversed or halted by the implementation of a GFD (Bathrellou et al., 2018).

Complications and comorbidities in patients with NCGS are far less common than those of CD (Bathrellou et al., 2018). Cardiovascular disease remains a risk factor for both CD and NCGS patients due to the lack of whole grain consumption when on a GFD. Nutritional deficiencies may also be a risk factor due to the increased permeability in the intestinal lining, but this is reversible with implementation of a GFD. Interestingly, the most closely associated comorbidities of NCGS are psychiatric in nature. Whether this is a causal or correlational relationship, however, is unknown. As mentioned before, since NCGS is a relatively new diagnosis, data surrounding the long-term effects and complications is unavailable (Bathrellou et al., 2018).

Conclusion

Gluten is becoming an increasingly popular topic among consumers of the Western diet. Some people ask what it is; others assume that all of their health issues can be solved by adopting a GFD. However, self-diagnosis and/or confusion of CD and NCGS can be harmful to the patient in a variety of ways. Careless adoption of a GFD can actually lead to more nutritional problems than the well-meaning patient started with in the first place (Reilly, 2016). Gluten-free

alternative foods can be higher in fat, sugar, and some toxins in order to make them taste as good as their gluten-containing counterparts, which can ultimately lead to a variety of health problems in the patient. However, the GFD is safe and extremely effective as a treatment for CD and NCGS (Reilly, 2016). These two diseases are similar in many ways but have enough difference between them to be clinically significant, which is why differentiation between the two is essential to specific, effective treatment for each disease. Both of these diseases are expected to continue to increase in prevalence (Catassi et al., 2010). The reason for this is not clear, but it does warrant further research into the exact mechanisms of pathogenesis of each in order to treat patients more effectively. CD and NCGS both affect the small intestinal lining with varying degrees of damage and/or villous atrophy following the ingestion of gluten proteins from cereals such as wheat, barley, and rye (Leonard et al., 2017). However, it is still unclear whether gluten is the true trigger for NCGS, as removal of wheat ATIs and FSAPs also alleviate NCGS associated symptoms (Leccioli et al., 2017). Since these pathologies are so similar, the diagnostic process is largely the same (Elli et al., 2015). NCGS is only clinically diagnosed after testing for CD and other gluten and wheat-related disorders is negative. Treatment for these diseases is a large area of research that has not been adequately investigated due to the efficacy of the GFD (Allen, 2016). However, treatments aimed at detoxifying gluten have the most potential to be beneficial in addition to the GFD (Sample et al., 2017). More effective treatments will, in theory, lead to higher compliance among CD and NCGS patients, which will decrease the incidence rates of common complications, risk factors, and comorbidities.

References

Allen, P. J. (2016). Gluten-related disorders: Symptoms, diagnosis and management. *Primary Health Care (2014+); London*, *26*(5), 20. http://dx.doi.org/10.7748/phc.26.5.20.s28

Bathrellou, E., Kontogianni, M. D., & Panagiotakos, D. B. (2018). Celiac disease and non-celiac gluten or wheat sensitivity and health in later life: A review. *Maturitas*, *112*, 29–33. https://doi.org/10.1016/j.maturitas.2018.03.014

Biesiekierski, J. R. (2017). What is gluten? *Journal of Gastroenterology and Hepatology*, *32*(S1), 78–81. https://doi.org/10.1111/jgh.13703

Bischoff, S. C., Barbara, G., Buurman, W., Ockhuizen, T., Schulzke, J.-D., Serino, M., Tilg, H., Watson, A., & Wells, J. M. (2014). Intestinal permeability – a new target for disease prevention and therapy. *BMC Gastroenterology*, *14*(1), 1–25. https://doi.org/10.1186/s12876-014-0189-7

Bouziat, R., Hinterleitner, R., Brown, J. J., Stencel-Baerenwald, J. E., Ikizler, M., Mayassi, T., … Aravamudhan, P. (2017). Reovirus infection triggers inflammatory responses to dietary antigens and development of celiac disease. *Science*, *356*(6333), 44–50. https://doi.org/10.1126/science.aah5298

Cabanillas, B. (2019). Gluten-related disorders: Celiac disease, wheat allergy, and nonceliac gluten sensitivity. *Critical Reviews in Food Science and Nutrition*, *0*(0), 1–16. https://doi.org/10.1080/10408398.2019.1651689

Cagno, R. D., Angelis, M. D., Pasquale, I. D., Ndagijimana, M., Vernocchi, P., Ricciuti, P., Gagliardi, F., Laghi, L., Crecchio, C., Guerzoni, M. E., Gobbetti, M., & Francavilla, R. (2011). Duodenal and faecal microbiota of celiac children: Molecular, phenotype and

metabolome characterization. *BMC Microbiology*, *11*(1), 1–21. https://doi.org/10.1186/1471-2180-11-219

Capacci, S., Leucci, A. C., & Mazzocchi, M. (2018). There is no such thing as a (gluten-)free lunch: Higher food prices and the cost for coeliac consumers. *Economics & Human Biology*, *30*, 84–91. https://doi.org/10.1016/j.ehb.2018.06.001

Chander, A. M., Yadav, H., Jain, S., Bhadada, S. K., & Dhawan, D. K. (2018). Cross-Talk Between Gluten, Intestinal Microbiota and Intestinal Mucosa in Celiac Disease: Recent Advances and Basis of Autoimmunity. *Frontiers in Microbiology*. Gale OneFile: Health and Medicine. http://link.gale.com/apps/doc/A560773588/HRCA?u=vic_liberty&sid=zotero&xid=ab975ffa

Collyer, E. M., & Kaplan, B. S. (2016). Nonceliac gluten sensitivity: An approach to diagnosis and management. *Current Opinion in Pediatrics*, *28*(5), 638–643. https://doi.org/10.1097/MOP.0000000000000392

Cook, L., Munier, C. M. L., Seddiki, N., van Bockel, D., Ontiveros, N., Hardy, M. Y., … Kelleher, A. D. (2017). Circulating gluten-specific FOXP3+CD39+ regulatory T cells have impaired suppressive function in patients with celiac disease. *Journal of Allergy and Clinical Immunology*, *140*(6), 1592-1603.e8. https://doi.org/10.1016/j.jaci.2017.02.015

Coqueiro, A. Y., Bonvini, A., Tirapegui, J., & Rogero, M. M. (2017). Probiotics supplementation as an alternative method for Celiac disease treatment. *International Journal of Probiotics & Prebiotics, 12*(1), 23-. Health Reference Center Academic.

Di Sabatino, A., Brunetti, L., Carnevale Maffè, G., Giuffrida, P., & Corazza, G. R. (2013). Is it worth investigating splenic function in patients with celiac disease? *World Journal of Gastroenterology : WJG*, *19*(15), 2313–2318. https://doi.org/10.3748/wjg.v19.i15.2313

Dieterich, W., & Zopf, Y. (2019). Gluten and FODMAPS—Sense of a Restriction/When Is Restriction Necessary? *Nutrients; Basel*, *11*(8), 1957. http://dx.doi.org.ezproxy.liberty.edu/10.3390/nu11081957

Dieterich, W., Schuppan, D., Schink, M., Schwappacher, R., Wirtz, S., Agaimy, A., Neurath, M. F., & Zopf, Y. (2019). Influence of low FODMAP and gluten-free diets on disease activity and intestinal microbiota in patients with non-celiac gluten sensitivity. *Clinical Nutrition*, *38*(2), 697–707. https://doi.org/10.1016/j.clnu.2018.03.017

Discepolo, V., & Guandalini, S. (2017). Celiac Disease Treatment: Is It the Chicken or the Egg Yolk? *Digestive Diseases and Sciences; New York*, *62*(5), 1113–1115. http://dx.doi.org/10.1007/s10620-017-4489-y

Elli, L., Branchi, F., Tomba, C., Villalta, D., Norsa, L., Ferretti, F., Roncoroni, L., & Bardella, M. T. (2015). Diagnosis of gluten related disorders: Celiac disease, wheat allergy and non-celiac gluten sensitivity. *World Journal of Gastroenterology : WJG*, *21*(23), 7110–7119. https://doi.org/10.3748/wjg.v21.i23.7110

Fasano, A., & Catassi, C. (2012). Celiac Disease. *The New England Journal of Medicine; Boston*, *367*(25), 2419–2426.

Gaesser, G. A., & Siddhartha, A. S. (2012). Gluten-Free Diet: Imprudent Dietary Advice for the General Population? *Journal of the Academy of Nutrition and Dietetics*, *112*(9), 1330–1333. https://doi.org/10.1016/j.jand.2012.06.009

Green, P. H., Lebwohl, B., & Greywoode, R. (2015). Celiac disease. *Journal of Allergy and Clinical Immunology; St. Louis*, *135*(5), 1099–1106. http://dx.doi.org/10.1016/j.jaci.2015.01.044

Hoffmann, H. J., Valovirta, E., Pfaar, O., Moingeon, P., Schmid, J. M., Skaarup, S. H., … Sørensen, P. (2017). Novel approaches and perspectives in allergen immunotherapy. *Allergy*, *72*(7), 1022–1034. https://doi.org/10.1111/all.13135

Kabbani, T. A., Vanga, R. R., Leffler, D. A., Villafuerte-galvez, J., Pallav, K., Hansen, J., Mukherjee, R., Dennis, M., & Kelly, C. P. (2014). Celiac Disease or Non-Celiac Gluten Sensitivity? An Approach to Clinical Differential Diagnosis. *The American Journal of Gastroenterology; Cambridge*, *109*(5), 741–746. http://dx.doi.org/10.1038/ajg.2014.41

Kagnoff, M. (n.d.). Overview and pathogenesis of celiac disease. *Gastroenterology*, *128*(4), S10–S18. https://doi.org/10.1053/j.gastro.2005.02.008

Kupfer, S. S., & Jabri, B. (2012). Pathophysiology of Celiac Disease. *Gastrointestinal Endoscopy Clinics of North America*, *22*(4), 639–660. https://doi.org/10.1016/j.giec.2012.07.003

Lebwohl, B., Ludvigsson, J. F., & Green, P. H. R. (2015). Celiac disease and non-celiac gluten sensitivity. *BMJ : British Medical Journal (Online); London*, *351*. http://dx.doi.org/10.1136/bmj.h4347

Leccioli, V., Oliveri, M., Romeo, M., Berretta, M., & Rossi, P. (2017). A New Proposal for the Pathogenic Mechanism of Non-Coeliac/Non-Allergic Gluten/Wheat Sensitivity: Piecing Together the Puzzle of Recent Scientific Evidence. *Nutrients; Basel*, *9*(11), 1203. http://dx.doi.org/10.3390/nu9111203

Leonard, M. M., Sapone, A., Catassi, C., & Fasano, A. (2017). Celiac Disease and Nonceliac Gluten Sensitivity: A Review. *JAMA*, *318*(7), 647–656. https://doi.org/10.1001/jama.2017.9730

Malamut, G., & Cellier, C. (2015). Complications of coeliac disease. *Best Practice & Research*, *29*(3), 451–458. ProQuest Central; ProQuest Central. https://doi.org/10.1016/j.bpg.2015.05.005

Malamut, G., El Machhour, R., Montcuquet, N., Martin-Lannerée, S., Dusanter-Fourt, I., Verkarre, V., … Meresse, B. (2010). IL-15 triggers an antiapoptotic pathway in human intraepithelial lymphocytes that is a potential new target in celiac disease–associated inflammation and lymphomagenesis. *The Journal of Clinical Investigation*, *120*(6), 2131–2143. https://doi.org/10.1172/JCI41344

McLaughlin, J. (2009). Gastrointestinal physiology. *Surgery (Oxford)*. *27*, 225-230.

Meresse, B., Korneychuk, N., Malamut, G., & Cerf-Bensussan, N. (2015). Interleukin-15, a master piece in the immunological jigsaw of celiac disease. *Digestive Diseases*, *33*(2), 122–130. https://doi.org/10.1159/000369521

Mesnage, R., & Antoniou, M. N. (2017). Facts and Fallacies in the Debate on Glyphosate Toxicity. *Frontiers in Public Health*, *5*. https://doi.org/10.3389/fpubh.2017.00316

Murray, J. A., Frey, M. R., & Oliva-Hemker, M. (2018). Celiac Disease. *Gastroenterology*, *154*(8), 2005–2008. https://doi.org/10.1053/j.gastro.2017.12.026

Reilly, N. R. (2016). The Gluten-Free Diet: Recognizing Fact, Fiction, and Fad. *The Journal of Pediatrics*, *175*, 206–210. https://doi.org/10.1016/j.jpeds.2016.04.014

Rigamonti, A., Magi, S., Venturini, E., Morandi, L., Ciano, C., & Lauria, G. (2007). Celiac disease presenting with motor neuropathy: Effect of gluten free-diet. *Muscle & Nerve*, *35*(5), 675–677. https://doi.org/10.1002/mus.20727

Rubio-Tapia, A., & Murray, J. A. (2007). The liver in celiac disease. *Hepatology*, *46*(5), 1650–1658. https://doi.org/10.1002/hep.21949

Sample, D. A., Sunwoo, H. H., Huynh, H. Q., Rylance, H. L., Robert, C. L., Xu, B., Kang, S. H., Gujral, N., & Dieleman, L. A. (2017). AGY, a Novel Egg Yolk-Derived Anti-gliadin Antibody, Is Safe for Patients with Celiac Disease. *Digestive Diseases and Sciences; New York*, *62*(5), 1277–1285. http://dx.doi.org.ezproxy.liberty.edu/10.1007/s10620-016-4426-5

Samsel, A., & Seneff, S. (2013). Glyphosate, pathways to modern diseases II: Celiac sprue and gluten intolerance. *Interdisciplinary Toxicology*, *6*(4), 159–184. https://doi.org/10.2478/intox-2013-0026

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. https://doi.org/10.1371/journal.pone.0053414

Wood, R. A. (2016). Food allergen immunotherapy: Current status and prospects for the future. *Journal of Allergy and Clinical Immunology*, *137*(4), 973–982. https://doi.org/10.1016/j.jaci.2016.01.001

Yasuda, T., Ura, T., Taniguchi, M., & Yoshida, H. (2016). Intradermal delivery of antigens enhances specific igg and diminishes ige production: Potential use for vaccination and

allergy immunotherapy. *PLOS ONE, 11*(12), e0167952. https://doi.org/10.1371/journal.pone.0167952

Yen, T.-H., & Wright, N. A. (2006). The gastrointestinal tract stem cell niche. *Stem Cell Reviews*, *2*(3), 203–212. https://doi.org/10.1007/s12015-006-0048-1

Zugna, D., Richiardi, L., Akre, O., Stephansson, O., & Ludvigsson, J. F. (2010). A nationwide population-based study to determine whether coeliac disease is associated with infertility. *Gut*, *59*(11), 1471. ProQuest Central; ProQuest Central; SciTech Premium Collection. https://doi.org/10.1136/gut.2010.219030