Dietary Proteins and Functional Gastrointestinal Disorders

Erica Boettcher, MD[1] and Sheila E. Crowe, MD[1]

Food intolerance is a common complaint amongst patients with functional gastrointestinal (GI) disorders (FGIDs), including those with irritable bowel syndrome (IBS), functional dyspepsia, as well as gastroesophageal reflux disease. Although there has been a longstanding interest in the possible role of food allergy in IBS, there are limited data supporting the association. However, the prevalence of food allergy is sufficiently high that patients with FGID may also have food allergies or hypersensitivities. Food intolerances or sensitivities are reactions to foods, which are not due to immunological mechanisms. Lactose intolerance is common in the general population and can mimic symptoms of FGID or coexist with FGID. As discussed in other articles in this series, other carbohydrate intolerances may be responsible for symptom generation in patients with IBS and perhaps other FGIDs. There is a great interest in the role of a major dietary protein, gluten, in the production of symptoms that are very similar to those of patients with celiac disease without the enteropathy that characterizes celiac disease. Emerging research into a syndrome known as nonceliac gluten sensitivity suggests a heterogeneous condition with some features of celiac disease but often categorized as FGIDs, including IBS. This article summarizes the role of dietary proteins in the symptoms and pathophysiology of FGIDs.

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INTRODUCTION

Conventional therapies for functional gastrointestinal (GI) disorders (FGID) have mainly focused on relief of symptoms, such as pain, diarrhea, and constipation. Many patients with FGID believe that specific foods or dietary components have a key role in inducing symptoms, but the mechanisms for food-specific GI symptoms are not well elucidated.

A number of observations support a role for food intake in the pathogenesis of FGID symptoms, both in the irritable bowel syndrome (IBS) and functional dyspepsia. The majority of patients report that food intake induces symptoms (1,2). Many patients report problems with specific foods, and a myriad of dietary interventions have been proposed as therapeutic approaches in alleviating patients' symptoms (3–6). Although these observations suggest a role of diet in the pathogenesis and treatment of FGID symptoms, the underlying mechanisms for food-specific intolerances or sensitivities are poorly understood and well-conducted, high-quality studies are lacking.

Any abnormal reaction resulting from the ingestion of a food is considered an adverse food reaction (see **Figure 1**). Such reactions may be the result of food allergies or food sensitivities, or intolerances. Food allergies are adverse health effects that arise from specific immune responses occurring reproducibly on exposure to a specific food (7). Foods or food components that elicit an adverse reaction but have no established immunologic mechanism are termed food sensitivities.

FOOD ALLERGIES

Food allergens are usually proteins, which are biopolymers built of various combinations of 20 different naturally occurring amino acids. The primary dietary sources of proteins are muscle, milk, egg and plant proteins, and within each source is a complex mixture of proteins. Muscle proteins originate from meat products, including red meat, fish, and poultry. Milk proteins are represented by two major groups: caseins and whey proteins. Egg proteins are morphologically divided into proteins of egg white (albumen) and yolk. Plant proteins include cereal, pulse, and legume proteins (8). Proteins are hydrolyzed by a range of peptidases, each with specificity for peptide bonds between different amino acids. Endopeptidases attack internal bonds and liberate large peptide fragments, whereas exopeptidases cleave off one amino acid at a time from either the carboxyl or the amino terminus. The final products are free amino acids and di- and tripeptides, which are absorbed by epithelial cells of the small intestine.

Along with processing protein and other food components into a form that can be absorbed and utilized for energy and cell growth, the GI tract must discriminate between harmful and

[1]Division of Gastroenterology, Department of Medicine, University of California San Diego, San Diego, California, USA. **Correspondence:** Sheila E. Crowe, MD, Division of Gastroenterology, Department of Medicine, University of California San Diego, 9500 Gilman Drive, San Diego, California 92093-0063, USA. E-mail: secrowe@ucsd.edu

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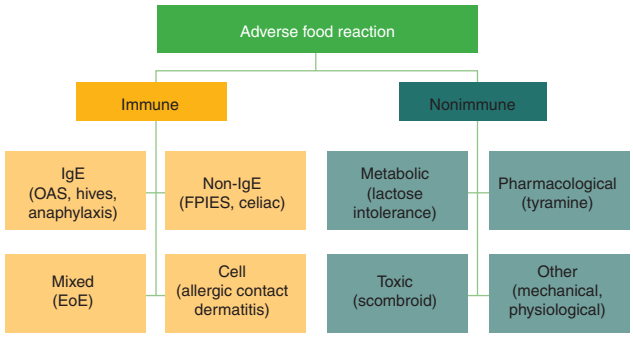

Figure 1. Categorization of adverse reactions to food. Any abnormal reaction resulting from the ingestion of a food is considered an adverse food reaction. Such reactions may be immune-mediated, termed food allergy, or nonimmune mediated, termed food sensitivity or food intolerance. The term food allergy comprises clinical conditions associated with altered immunologic reactivity that may be IgE-mediated or non-IgE-mediated. Examples of IgE-mediated responses include oral allergy syndrome (OAS), hives, and anaphylaxis. Among the non-IgE-mediated responses are food protein-induced enterocolitis syndrome (FPIES), eosinophilic esophagitis (EoE), and eosinophilic gastroenteritis, as well as celiac disease. Foods or food components that elicit an adverse reaction but have no established immunologic mechanism are termed food sensitivities. Among the mechanisms for food sensitivity are food toxicity, as well as pharmacological, metabolic, physiological, and psychological food sensitivities. Modified from NIAID-Sponsored Expert Panel (7).

harmless foreign proteins. Every day, the GI tract is exposed not only to commensal bacteria of the GI tract and food but also to potentially harmful bacteria and other pathogens. The GI mucosa has an extensive immune system, which, together with mechanical barriers, has a pivotal role in defending the host against possible offending agents (9).

In a healthy gut, the mucosal immune system interacts with commensal bacteria and dietary antigens without generating an inflammatory response and can mount an appropriate host response to pathogenic microorganism exposure. In an intact mucosal barrier, only small quantities of antigen or pathogen cross beyond the epithelium and a mechanism exists for downregulation of the immune response to the agents that do cross, leading to what is termed "oral tolerance". The two divisions of the GI immune system that allow this response to occur are innate and adaptive immunity (9).

Innate immunity is the first line of defense and is closely linked to the digestive and absorptive processes of the GI tract. It can be further divided into two forms of response, nonimmunologic and immunologic. Nonimmunological mechanisms protecting the mucosa against foreign antigen contact consist various physiologic chemicals, antimicrobial elements, and mechanical processes, including peristalsis and an intact epithelial barrier provided by tight junctions. Immunological components include the complement system, phagocytes, and natural killer cells. The adaptive immune response is mediated through both humoral and cellular immunity. Humoral immunity, via B-lymphocytes, results in antibody production to defend against harmful pathogens. Cellular immunity, via T lymphocytes, protects against untoward intracellular events (9).

Developmental immaturity in infants reduces the efficiency of the mucosal barrier, and likely has a major role in the increased prevalence of GI infections and food allergy seen in the first few years of life. In addition, in a susceptible host, a failure to develop or a breakdown in oral tolerance may result in sensitization to food allergens. The eight major allergens in many developed countries include peanuts, tree nuts, cow's milk protein, eggs, wheat, soy protein, shellfish, and fish. These eight foods/groups account for 90% of food allergies in the United States (10).

The term "allergy" includes clinical conditions associated with altered immunologic reactivity that may be IgE-mediated or non-IgE-mediated. IgE is a unique class of immunoglobulin that mediates an immediate allergic reaction. The most established abnormal immunologic reactions to food are caused by allergen-specific IgE-mediated, so-called "immediate hypersensitivity" reactions. These reactions are involved in the pathogenesis of many cases of asthma, rhinitis, urticaria, atopic dermatitis, and GI adverse food reaction. Delayed reactions following immediate IgE-mediated hypersensitivity can also occur and are characterized by an enhanced cell infiltration of the tissue with inflammatory cells and subsequent tissue damage (11).

There are additional immunologic, non-IgE-induced mechanisms, including eosinophilic GI disorders (eosinophilic esophagitis, eosinophilic enteritis, eosinophilic colitis, and eosinophilic gastroenteritis), food protein enteropathy, enterocolitis and proctitis, and celiac disease, which are also considered to be food allergies. In these conditions, sensitization to food protein cannot always be demonstrated based on an allergen-specific IgE. The diagnosis of non-IgE-mediated food allergy is based on signs and symptoms occurring reproducibly on exposure to food, resolution of those signs and symptoms with specific food avoidance, and histologic evidence of an immunologically mediated process.

FOOD INTOLERANCES OR SENSITIVITIES

Foods or food components that elicit an adverse reaction but have no established immunologic mechanism are termed food sensitivities (recommended or preferred term in the United States (7)) or food intolerance. Among the mechanisms for different forms of food sensitivity are food toxicity, as well as pharmacological, metabolic, physiological and psychological food sensitivities. Those with no established mechanism are "idiosyncratic" food sensitivities (11). **Table 1** summarizes the major categories of food sensitivities.

Food toxicity results from microbial contamination of food causing GI symptoms generally from preformed toxins, such as staphylococcal enterotoxin, or replication of enteric pathogens, such as *Shigella*, *Salmonella*, *Campylobacter* or *Escherichia coli*. These reactions usually do not recur and have typical presentations (11).

Pharmacological food sensitivities are reactions to food due to chemical components in foods and food additives, and most cause symptoms outside of the GI tract. Examples include histamine found in Swiss cheese, tuna, and other scombroid fish, causing headaches and diffuse erythema of the skin, and glutamate, which can cause a syndrome of warm sensation, chest tightness, headache, and gastric discomfort (11).

Table 1. Categories of sensitivities or intolerances to food

Category	Examples
Food toxicity	Effects of food-borne pathogens, including microbial toxins
Pharmacological	Adverse reactions to histamine in foods, such as scombroid fish poisoning
Metabolic	Lactose intolerance
Physiological	Consequences of ingestion or digestion of certain foods, such as fatty foods, legumes, and many other foods
Psychological	Eating disorders, aversion to food because of taste, texture, and other mechanisms
Idiosyncratic	Unpredictable and unexplained reactions to foods; e.g., NCGS

Among the metabolic food sensitivities, the most common is lactose intolerance. Primary lactose intolerance is most commonly due to declining levels of intestinal lactase activity in later childhood and adulthood, but can rarely manifest as a congenital deficiency. Symptoms are dose dependent and include bloating, flatulence, and diarrhea. Secondary lactase deficiency can also be seen in viral gastroenteritis, radiation enteritis, Crohn's disease, and celiac disease among others.

Physiologic food sensitivities result from physiological reactions to food components or additives. An example is the starch found in legumes, which serves as a substrate for gas production by colonic flora. Psychological food sensitivities include taste aversion, texture aversion, fear of the consequences of eating, conditioned responses, eating disorders, and those secondary to a traumatic experience (e.g., from abuse), neglect, or food poisoning. Idiosyncratic food sensitivities have no established mechanism and are, in general, controversial. Among the most common food proteins that have been reported to cause idiosyncratic food sensitivities are gluten and cow's milk.

CELIAC DISEASE AND NONCELIAC GLUTEN SENSITIVITY

Gluten is a complex of water-soluble proteins from wheat, rye, and barley (12). "Gluten-related disorders" is a term used to describe all conditions related to gluten, including celiac disease, dermatitis herpetiformis, gluten ataxia, and nonceliac gluten sensitivity (NCGS) (13).

Celiac disease is characterized by chronic inflammation of the proximal small intestinal mucosa that heals when foods containing gluten are excluded from the diet and returns when foods containing gluten are reintroduced. Gluten contains the storage proteins derived from wheat, barley, and rye. These proteins are enriched in glutamines and prolines, and undergo partial digestion in the upper GI tract, which results in various native peptide derivatives. The specific peptides that can elicit an immune response vary and occur throughout the storage proteins of all the three grains. These immunogenic peptides are resistant to digestion by GI proteases and can be taken up intact in the small intestine by paracellular and transcellular routes into the lamina propria where they interact with immune effector cells (14). Most patients who develop celiac disease make tissue transglutaminase (tTG) or transglutaminase 2 autoantibodies. Transglutaminase 2 acts to deamidate glutamine to negatively charged glutamic acid residues. The deamidated gliadin peptides then bind to products of heterodimeric human leukocyte antigen (HLA)-class II genes *HLA-DQ2* or *HLA-DQ8*, and their binding results in gluten-specific CD4+Th1 T-cell activation and an immune response that causes the intestinal injury characteristic of celiac disease. The histopathologic manifestations include intraepithelial lymphocytosis, lamina propria inflammation and varying degrees of villous atrophy (15).

The available screening tests for celiac disease include antigliadin antibodies, endomysial antibodies (EMA), tTG, and deamidated gliadin peptide). IgG antigliadin antibodies, deamidated gliadin peptide, and tTG antibodies are also available, but are largely used in the setting of IgA deficiency. Overall, the tTG IgA is the recommended test to screen and the inclusion of other tests in the panel adds little to the sensitivity, but increases the economic cost to specificity if a positive result leads to further testing (14). The diagnosis can be suggested by detecting tTG IgA in serum and confirmed by small intestinal biopsy. In spite of the high sensitivity of celiac-disease serology up to 10% of celiac disease cases are seronegative. This false-negative rate is only, in part, due to IgA deficiency, the most common hereditary immunodeficiency in the general population (1:400–1:700), and is variably reported as increased amongst patients with celiac disease. Thus, if there are symptoms that warrant further assessment, such as unexplained diarrhea or iron deficiency anemia, then endoscopy with intestinal biopsies should be performed. Another important component of diagnostic testing in celiac disease is the use of *HLA-DQ2/DQ8* testing, as nearly 100% of patients with celiac disease carry the *DQ2* or *DQ8* alleles as compared with 30–35% of the general US population. As the presence of those alleles provides close to 100% sensitivity for celiac disease, the test is only useful in excluding celiac disease, given a very high negative predictive value for the disease.

Once a diagnosis of celiac disease is made, the benefits of adhering to a gluten-free include improvement of symptoms as its primary and most common benefit, and may also reduce overall cancer risk (16), improve quality of life (17), ameliorate osteoporosis, correct iron deficiency, and improve unexplained infertility and pregnancy outcomes (18).

In contrast to celiac disease, NCGS, also described in the literature as gluten sensitivity, gluten hypersensitivity, gluten intolerance, and nonceliac gluten intolerance (19), is one or more of a variety of symptomatic manifestations precipitated by ingestion of gluten-containing foods in individuals in whom celiac disease and wheat allergy have been excluded (13,19–21). The diagnosis is based largely on an association between the ingestion of gluten and the development of adverse symptoms. NCGS can be characterized by gastrointestinal symptoms, such as diarrhea, abdominal discomfort, or pain, and bloating and flatulence, or extraintestinal manifestations, such as headache, lethargy, attention-deficit/hyperactivity disorder, ataxia, or recurrent oral ulceration, which

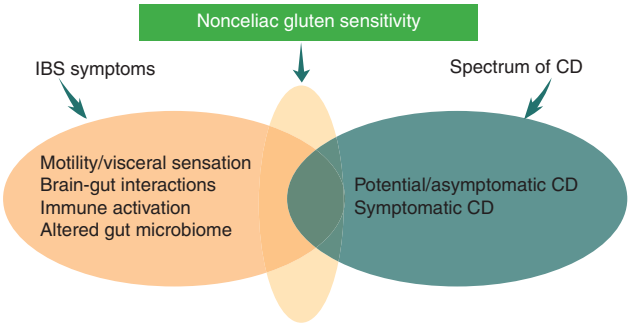

Figure 2. Is it IBS, celiac disease, or something in between? Nonceliac gluten sensitivity (NCGS) encompasses a collection of medical conditions in which gluten leads to an adverse food reaction, which can be clinically indistinguishable from celiac disease (CD), but testing is negative or inconclusive. NCGS may be one of the underlying mechanisms for symptom generation in irritable bowel syndrome (IBS) and may not necessarily belong to the spectrum of CD. Modified from Verdu *et al.* (24).

improve or disappear after gluten withdrawal in patients in whom celiac disease and wheat allergy have been ruled out (22). As there is no known mechanism, there are currently no diagnostic criteria or serological testing available for the disorder. One study suggests that increased antigliadin IgG antibodies are often found in patients with NCGS (23).

Although there is no well-established mechanism for NCGS, the gluten-free diet has gained substantial popularity with the general public and this is reflected in the more than doubling of sales of gluten-free products since 2005. Projected US sales are expected to hit $1.68 billion and $3.38 billion globally by 2015 (Reuters online, 29 September 2011; http://www.reuters.com/article/2011/09/29/uk-food-glutenfree-idUSLNE78S00W20110929). Gluten-free products are now readily available in many geographic locations and several large US food-manufacturing companies now offer gluten-free alternatives.

Verdu *et al.* (24) describe NCGS as the "no man's land" between FGIDs and the spectrum of celiac disease, recognized neither by the FGID or celiac disease specialists at that time (see **Figure 2**). Some data suggests that a subset of patients labeled as NCGS may, in actual, belong to the spectrum of celiac disease, representing a milder form of celiac disease with abnormal serologic celiac antibodies, a fitting HLA haplotype, but nondiagnostic duodenal biopsies (25). Subtle immunopathological changes in the intestine exposed to gluten have been described that do not meet the criteria for celiac disease but typically occur in individuals that carry the same HLA genotypes associated with the disease. Pathologic changes that have been described include increased intraepithelial lymphocytosis (26), increased IgA deposition in the intestinal villi (27), changes in the microvillus border, and an increase in secreted antibodies directed against gliadin (28).

In support of a subset of NCGS belonging to the spectrum of celiac disease, Kaukinen *et al.* (29) studied 10 patients with celiac disease-like symptoms and Marsh 1 or 2 mucosal lesions, which correspond to increased intraepithelial lymphocytes without other small bowel architectural changes (Marsh 1) or plus crypt hyperplasia (Marsh 2). Of 10 patients maintained on gluten-free diet for 6–12 months, 8 patients had resolution of symptoms with statistically significant improvement of mucosal lesions. All of the patients who responded to the gluten-free diet were *HLA-DQ2* positive; serum EMA IgA was initially positive in 80% and tTG IgA was positive in 90%. When patients were maintained on the gluten-free diet, all antibody levels decreased to normal or remained only slightly elevated.

Similarly, Wahnscahaffe *et al.* (30), in a nonrandomized prospective study, investigated 102 patients with diarrhea-predominant IBS, who had normal biopsies or increased intraepithelial cell counts and negative celiac serologies. Fifty-eight percent of the IBS patients carrying *HLA-DQ2* alleles had positive IgA antigliadin or anti-tTG antibodies in duodenal aspirate and higher intraepithelial lymphocytes, compared with 15% of IBS patients with negative *HLA-DQ2*. Noting that the determination of antibodies in intestinal fluid is invasive, nonstandard, and probably not feasible as a routine test, they extended their earlier study to investigate serum IgG antibodies against gliadin or tTG in IBS patients and examined the sensitivity and specificity of these markers to predict the clinical response to a gluten-free diet (31). They evaluated 51 diarrhea-predominant IBS patients for clinical response to a gluten-free diet. *HLA-DQ2* genotype testing and baseline and interval serologic testing was performed, including serum antigliadin antibodies IgA and IgG. Small bowel biopsies were performed to evaluate for celiac disease. For IBS patients with abnormal celiac antibody tests, but nondiagnostic small bowel biopsy results, antigliadin antibodies IgG significantly decreased in only *HLA-DQ2*-positive patients after 6 months on a gluten-free diet ($P < 0.01$), with no significant change in tTG IgG concentrations. GI symptoms significantly improved on the gluten-free diet, and the effect was more pronounced in *HLA-DQ2*-positive patients ($P < 0.01$). The authors concluded that the presence of *HLA-DQ2* and its association of clinical response to a gluten-free diet was 92% sensitive and 52% specific. Its absence had a 94% negative predictive value.

Aside from the celiac disease spectrum, many potential mechanisms for NCGS have surfaced in recent years. One of the proposed mechanisms is an innate immune reaction. Although the typical aspects of overt inflammation or mucosal architecture distortion are absent in patients with IBS, some data suggests mild activation of the immune system, both locally in the intestinal mucosa and systemically in plasma, serum, and peripheral blood mononuclear cells. It is contested whether these mild immune abnormalities are relevant to symptom manifestation, but some studies have shown that mucosal or luminal mediators obtained from patients with IBS, but not controls, evoked abnormal functional responses in enteric and sensory nerves (32), and disrupted the intestinal barrier integrity of recipient laboratory animals, isolated rodents (33), or human tissues or cell culture (34). The implication of intestinal immune activation in the pathogenesis of IBS is supported by the development of IBS symptoms in subjects involved in an episode of acute infectious gastroenteritis, the so-called post-infectious IBS. It is conceivable that other environmental triggers, such as gluten, may result in abnormal gut immune function leading to IBS.

In a study by Sapone *et al.* of 13 celiac disease, 11 NCGS and 7 control patients, NCGS patients had a number of CD3+ intraepithelial lymphocytes intermediate between celiac disease patients and controls in the context of conserved villous architecture. *IL-17A* gene expression in biopsy specimens was significantly elevated in active celiac patients compared with NCGS and control patients and the level of variance was also statistically significant ($P<0.025$). They concluded that celiac disease and NCGS are distinct entities and that the immune system deals with gluten in different ways, possibly depending on the genetic makeup of the subject (35).

In a more recent study, Sapone *et al.* (35) evaluated 26 patients with NCGS, 42 with celiac disease, and 39 controls, and found that patients with NCGS had significantly lower intestinal permeability measured by urinary lactulose/mannitol ratio as compared with celiac disease or control patients ($P=0.030$). Relative to controls, adaptive immunity markers interleukin-6 ($P=0.012$) and interleukin-21 ($P=0.057$) were expressed at higher levels in celiac disease but not in NCGS, whereas expression of the innate immunity marker Toll-like receptor-2 was increased in NCGS but not in celiac disease ($P=0.029$). Expression of the T-regulatory cell marker FOXP3 was significantly reduced in NCGS relative to controls ($P=0.032$) and celiac disease patients ($P=0.029$). The authors concluded that NCGS and celiac disease are different clinical syndromes, and that NCGS may be associated with gluten-induced activation of innate, rather than adaptive, immune responses in the absence of detectable changes in mucosal barrier function (36).

Another proposed mechanism for NCGS is the opioid hypothesis, as it has been established that peptides with opioid activity are found in pepsin hydrolysates of wheat gluten. The opioid activity of these peptides was demonstrated by the use of several bioassays, including naloxone-reversible inhibition of adenylate cyclase in homogenates of neuroblastoma X-glioma hybrid cells, naloxone-reversible inhibition of electrically stimulated contractions of the mouse vas deferens, and displacement of H3 dihydromorphine and H3-Tyr, DAla2 met-enkephalin amide from rat brain membranes, suggesting that they may be of physiological importance (37).

Yet another proposed mechanism is the "leaky gut" hypothesis. There is evidence supporting that tight junctions, once regarded as static structures, are in fact dynamic and they readily adapt to a variety of developmental (38), physiological (39), and pathological (40–44) circumstances. For instance in celiac disease, immune responses are initiated when immunogenic, incompletely digested gluten peptides gain entry into the lamina propria of the small intestine by transcellular transport and through the paracellular space between epithelial cells. Paracellular transport of gluten peptides may occur in the setting of increased paracellular permeability in patients with celiac disease due to gliadin-induced innate and adaptive immune responses (45–47) and subsequent tight junction disassembly (47–49). In contrast, there is no experimental data to suggest that intestinal permeability is altered in patients with NCGS. There is, however, some data reporting increased intestinal permeability in post-infectious IBS (50–52).

Other mechanisms have been proposed for NCGS, although there is scant scientific literature supporting the hypotheses of gluten toxicity, immune complex-mediated mechanisms, and autoimmunity. Alternatively, a component of wheat, aside from gluten, could be responsible for some of the symptomatic responses, as wheat starch itself is a highly fermentable substrate and its interaction with bacteria in the colon can lead to gas production and production of short-chain fatty acids, which in patients with abnormal motility and visceral hypersensitivity could lead to intestinal symptoms. Finally, NCGS may only be apparent and caused by the nocebo effect of wheat or gluten ingestion (22).

In an attempt to shed light on the role of wheat ingestion in the development of GI symptoms in IBS patients, Carroccio *et al.* (53) performed a retrospective analysis of 276 patients with an IBS-like clinical presentation, who had received a diagnosis of "wheat sensitivity" on the basis of a double-blind placebo-controlled (DBPC) challenge. Inclusion criteria for patients included normal duodenal biopsy, negative serum tTG and EMA IgA antibodies, negative skin prick tests, and serum-specific IgE antibodies, and those who had a resolution of symptoms on a wheat-free diet and reappearance on DBPC wheat challenge.

When enrolled in the study, patients who had self-restricted wheat were invited to ingest at least 30 g of wheat daily, and were observed for 2–4 weeks on a regular diet. Patients then underwent a standard elimination diet with exclusion of wheat, cow's milk, eggs, tomato, and chocolate. Patients who self-reported food sensitivity were also asked to avoid ingestion with the culprit food(s) causing symptoms. Food diaries were maintained to assess dietary intake and adherence to the diet. After 4 weeks, they underwent DBPC challenges with reintroduction of a single food at a time. In the case of wheat, the challenge was performed with capsules coded A or B containing wheat or xylose. Capsules A or B were given for 2 consecutive weeks, and then after a week of washout the patients received the other capsules for another 2 weeks. During all phases, the severity of symptoms was recorded. The challenges were considered positive if the same symptoms reappeared after their disappearance on elimination diet.

Of the 276 patients, 70 were diagnosed with "wheat sensitivity" alone, whereas 206 were diagnosed with multiple food sensitivities, including "wheat sensitivity". The symptom score for each symptom was significantly higher than at baseline from the first week on the DBPC wheat challenge ($P<0.0001$), and the values further increased at the end of the second week ($P<0.0001$). In the placebo group, there was no significant variation in the symptom score after weeks 1 and 2. The score on the wheat-containing diet was significantly higher than on placebo, both at the end of the first and second weeks into the DBPC challenge ($P<0.0001$). It is not clear from this study, however, if the reported sensitivity to wheat is due to gluten sensitivity or to another component of wheat, such as wheat starch.

In the first DBPC dietary rechallenge trial specifically investigating the role of gluten ingestion in IBS patients, Biesiekierski *et al.* (54) took 34 patients diagnosed with IBS by Rome III criteria, who had experienced symptom improvement with gluten-free diet for at least 6 weeks before study enrollment. Celiac disease had been excluded by either a negative *HLA-DQ2/HLA-DQ8* haplotype or a normal duodenal biopsy (Marsh 0). Patients with significant GI disease, such as cirrhosis or inflammatory bowel disease, and those

using nonsteroidal anti-inflammatory drugs or excessive alcohol, were excluded. Throughout the 6-week double-blind randomization phase of the trial, study participants were continued on a gluten-free diet and were provided study foods. The study food was randomized in 10 of the 34 patients to contain 16 g of gluten per day. The other 15 patients received gluten-free food, and preliminary testing in 10 healthy individuals revealed that the gluten-free foods could not be differentiated from gluten-containing foods on the basis of taste or texture. The gluten used in the study was free of fermentable oligo-, di-, monosaccharides, and polyols. The primary outcome was the proportion of patients answering "no" on more than half of the occasions at the end of each week to the question "Over the past week, were your symptoms adequately controlled?" Secondary outcomes, including bloating, abdominal pain, and satisfaction with stool consistency, nausea, and tiredness, were assessed using a 100-mm visual analog scale. At weeks 0 and 6, biomarkers, including high-sensitivity C-reactive protein and tTG IgA, whole gliadin IgA and IgG, and EMA, were measured. To assess intestinal permeability, a dual sugar test was performed, which relied on the measurement of urine lactulose/rhamnose. Finally, innate immunity and transepithelial migration of neutrophils were explored with fecal lactoferrin measurements.

It was found that a significantly greater proportion of patients in the gluten group compared with gluten-free group answered "no" to the primary outcome question (68% vs. 40%; $P<0.001$). Compared with the gluten group, those who remained gluten-free also reported improvements in pain ($P<0.016$), bloating ($P<0.031$), satisfaction with stool consistency ($P<0.024$), and tiredness ($P<0.001$), but showed no differences in wind ($P<0.053$) or nausea ($P<0.69$; see **Figure 3**). None of the patients had elevated tTG IgA or EMA at baseline, and there were no differences for whole-gliadin antibodies between the gluten and gluten-free groups. The results of celiac antibodies and other biomarkers after the dietary intervention were similar between the groups. Intestinal permeability as measured by urine lactulose-to-rhamnose ratio was unchanged by the dietary intervention. Fecal lactoferrin levels were persistently undetectable in all but one patient during the treatment period. There were no differences in the likelihood of symptomatic response in those with and without *HLA-DQ2/DQ8* alleles. The authors stated that these data support the existence of NCGS and concluded that gluten is associated with overall IBS symptoms, bloating, dissatisfaction with stool consistency, abdominal pain, and fatigue in a subset of patients.

Regarding the association between NCGS and functional dyspepsia, GERD, bloating, diarrhea, and constipation, there is currently very limited scientific literature available. As is the situation for IBS-like symptoms and celiac disease-like presentations, both proteins like gluten and other components of wheat, such as wheat starch, may be responsible for GI symptoms experienced in many FGIDs. Studies to date have substantial methodological shortcomings, and given the heterogenous nature of wheat or gluten sensitivity larger groups of patients will need to be examined to better understand the role of food components in IBS, functional dyspepsia, and many other FGIDs.

METHODS TO IDENTIFY PATIENTS WITH POSSIBLE NONCELIAC GLUTEN SENSITIVITY OR WHEAT INTOLERANCE

There are currently no defined diagnostic criteria and no underlying mechanism for NCGS, thus making the diagnosis challenging.

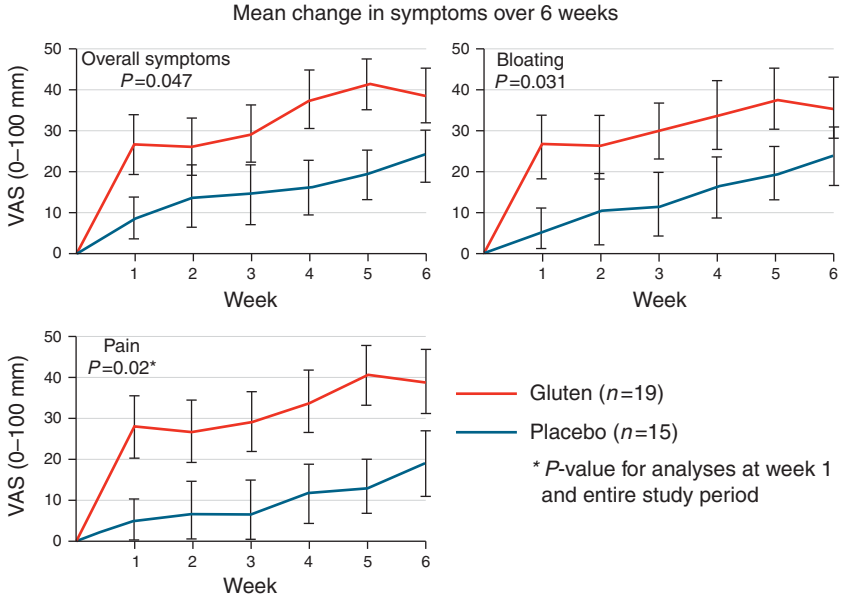

Figure 3. Gluten causes symptoms in IBS patients without celiac disease. Change in symptom severity from baseline in gluten ($n=19$) and placebo-treated ($n=15$) groups over a 6-week study period. Data shown represents mean change of symptoms using a visual analog scale (VAS) for subjects remaining on study therapy at each time point. *P*-values shown for overall symptoms and bloating represent the differences compared at week 1, which are statistically significant. *P*-value shown for abdominal pain represents the difference compared at week 1 and the entire study period, both of which are statistically significant. Modified from Biesiekierski *et al.* (54).

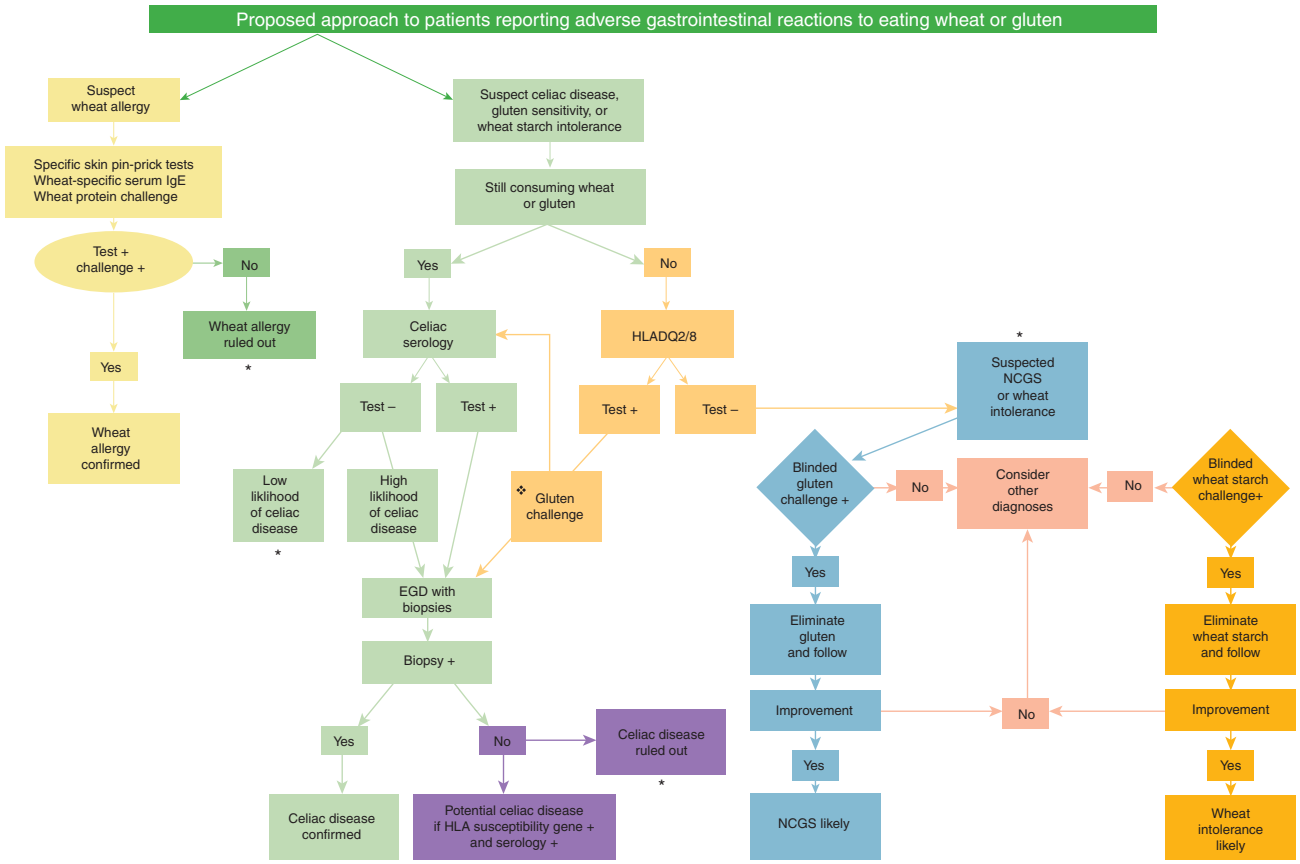

Figure 4. Proposed approach to patients reporting adverse gastrointestinal reactions to eating wheat or gluten. Proposed approach for the differential diagnosis of suspected wheat or gluten-related disorders, including wheat allergy, celiac disease (CD), non-celiac gluten sensitivity (NCGS), and wheat starch intolerance. The evaluation outlined is best conducted in centers with special interest and expertise in CD, food allergies, and/or food intolerances. In the case of suspected wheat allergy, skin pin-prick tests, wheat-specific serum IgE, and wheat protein challenge are conducted. If the IgE-based tests and challenge are positive, the diagnosis of wheat allergy is confirmed. If this evaluation is negative, wheat allergy is ruled out and other diagnoses should be considered (*proceed to suspected NCGS or wheat intolerance pathway). In suspected CD, NCGS, or wheat starch intolerance, if the patient is currently consuming wheat or gluten, CD is initially evaluated with serological testing (serum tissue transglutaminase (tTG) IgA, total IgA level, and potentially, gliadin peptide antibodies). If this is positive, esophagogastroduodenoscopy (EGD) is performed. If the tTG IgA is negative and there is a high likelihood of CD (otherwise unexplained diarrhea, iron deficiency, weight loss, and abdominal pain), EGD is also recommended. If the biopsy result is positive, the diagnosis of CD is confirmed. If the biopsy is negative and the patient has human leukocyte antigen (HLA) CD susceptibility genes, as well as tTG IgA positivity, the patient may have potential CD, but could also have NCGS. If the biopsy is negative and HLA gene testing is negative, CD is ruled out and one could proceed to assess for NCGS or wheat intolerance (*). If the patient is not currently consuming gluten, and CD, NCGS, and wheat intolerance are being considered, HLA DQ2/DQ8 testing is performed. If the patient has an HLA susceptibility gene, serological testing should be checked and, if positive, proceed to EGD with biopsy. If serology is not abnormal, a gluten challenge should be undertaken for up to 3 to 6 months if symptoms or serology do not become positive earlier, followed by EGD, including small intestinal biopsies. If the tTG IgA is negative and clinical suspicion for CD is low, then a diagnosis of either wheat intolerance or NCGS is suspected. At this point, it is recommended to perform a blinded wheat starch challenge or gluten challenge, ideally by the gold standard method, a double-blinded placebo-controlled (DBPC) challenge. In the first phase of the DBPC challenge, an elimination diet is initiated. This is followed by DBPC challenge, in the case of wheat intolerance, with wheat starch or placebo (xylose) administered as a capsule followed by washout period and crossover. In the case of NCGS, the challenge is identical, except with gluten capsules. If DBPC challenge is not possible, a nonblinded trial of wheat-starch-free or gluten-free diet followed by food challenge testing is initiated. If the DBPC challenge is positive, elimination of wheat starch or gluten is performed, and if there is an improvement wheat starch intolerance (or NCGS) is likely. If there is no improvement, other diagnoses are entertained, including small intestinal bacterial overgrowth and FGID, which can be associated with food sensitivities beyond wheat proteins or starch.

A proposed algorithm is as depicted below (see **Figure 4**). It should be noted that food challenges and allergy evaluation should be conducted in centers with expertise in food allergy and food intolerances.

The evidence for an elimination diet followed by food challenge testing comes from the initial observation by Jones *et al.* (55), of the role of food sensitivity in IBS (wheat, corn, diary, coffee, tea, and citrus). Since then, several studies have attempted to uncover this relationship further, although there are several methodological limitations, including trial design, inadequate patient selection, duration of elimination diet, and methods of food challenge employed (56). Prior studies have used IgG

enzyme-linked immunosorbent assay testing as the evidence of food allergy, but this type of testing is no longer accepted in the diagnosis of food hypersensitivity (7). In one recent study, Carroccio *et al.* (57) took blood from 120 IBS patients (Rome II) to analyze it for activation of basophils by food allergens (by flow cytometry), as well as total and food-specific IgE levels in serum to identify abnormal responses. Effects of elimination diets and double-blind food challenges were used as standards for food sensitivity. Patients completed a food sensitivity questionnaire and underwent open elimination diet for 4 weeks of cow's milk, wheat, egg, tomato, and chocolate. Responders went on to DBPC food challenges of milk vs. placebo for 2 weeks followed by wheat or placebo for 2 weeks. Of these patients, 36% improved with open elimination. Fifty-five percent of the IBS patients with food sensitivity had sensitivity to milk and/or wheat by DBPC food challenges. Forty-three percent had sensitivity to both, 7% to milk, and 5% to wheat. Problems appeared after a median of 3 days and 50% had to discontinue food challenges due to symptoms.

In this trial, patients both overestimated and underestimated food sensitivities in that 12/32 (38%) self-reporting food sensitivity improved on open elimination and reacted to DBPC challenge, and some patients who did not report sensitivity improved with the open challenge. Basophilic activity by flow cytometry was >85% accurate for food sensitivity despite the fact that they did/did not self-perceive the sensitivity.

COW'S MILK PROTEIN ALLERGY AND INTOLERANCE/SENSITIVITY

Cow's milk protein allergy (CMPA) affects 2–3% of young children and can present with a wide range of IgE-mediated and non-IgE-mediated clinical syndromes. Most children with CMPA have IgE-mediated allergy as a manifestation of their atopic constitution, with or without atopic dermatitis, asthma, or allergic rhinitis. Clinical symptoms often appear during the first months of life, usually within days to weeks of commencement of feeding with cow's milk-based formula. Patients with CMPA can present with a wide range of IgE-mediated and non-IgE-mediated syndromes, including the oral allergy syndrome, immediate hypersensitivity, eosinophilic esophagitis/gastroenteritis, dietary protein enteropathy, and dietary protein proctocolitis (58). The major cow's milk allergens are caseins and the whey proteins α-lactalbumin and β-lactoglobulin. Minor milk allergens include Bos d 6 (bovine serum albumin), lactoferrin, and immunoglobulins. In addition to CMPA, there are additional GI adverse food reactions to cow's milk, including lactose intolerance and intolerance of the long-chain triacylglycerol content of whole milk and cream, as well as items made from these dairy products.

For patients without cow's milk-specific IgE and lactose or triacylglycerol intolerance, the mechanism of cow's milk protein sensitivity is not understood, and the literature available for IBS, functional dyspepsia, GERD, bloating, diarrhea, and constipation in cow's milk protein sensitivity is very limited.

METHODS TO IDENTIFY PATIENTS WITH POSSIBLE COW'S MILK PROTEIN INTOLERANCE

Currently, there are no defined diagnostic criteria other than those established for cow's milk allergy in pediatric patients decades ago (59). The approach generally followed is to rule out CMPA, lactose intolerance, and triacylglycerol intolerance, via specific challenges. If the cow's milk protein challenge test is positive, cow's milk products are eliminated, and if improvement occurs, CMP allergy is likely. If elimination of lactose improves symptoms, lactose intolerance is likely. It is important to understand that not all diary products have lactose in sufficient amounts to cause symptoms. If drinking skim milk and avoiding cow's milk products with fat improve GI symptoms, then milk fat sensitivity is suggested. If elimination of all cow's milk products do not lead to an improvement of symptoms, consider other diagnoses such as overgrowth of bacteria of the small intestine, enteropathies, and FGIDs without specific food sensitivities.

FUTURE DIRECTIONS

We have focused on wheat and milk proteins as potential causes of food-induced symptoms in individuals with FGIDs. As is evident in the approach outlined in **Figure 4**, there are many components of wheat that can give rise to GI symptoms. Similarly, milk contains carbohydrates and fats in addition to milk proteins that can cause GI symptoms. In general, those who have FGIDs have increased sensitivity to food, additives, medications, and other ingested and external factors. Given the increasing reports of individuals with gluten sensitivity without celiac disease, this currently loosely defined condition requires further study to establish firm criteria for the diagnosis. Following this, studies are needed to understand the potential and likely multiple mechanisms by which gluten leads to GI symptoms. As the pathogenic mechanisms involved are elucidated, it will be possible to better define diagnostic criteria, develop appropriate tests, and evaluate the long-term consequences of avoiding the specific foods analogous to what is currently done for celiac disease.

CONFLICT OF INTEREST

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