

REVIEW ARTICLE

Non-celiac gluten sensitivity: clinical relevance and recommendations for future research

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Key Messages

- Non-Celiac Gluten Sensitivity (NCGS) is of increasing scientific interest in patients with unexplained gastrointestinal symptoms.
- A diagnosis of NCGS can only be made when celiac disease and wheat allergy are adequately excluded.
- NCGS is a heterogenous group of conditions best described by their improvement on exclusion of gluten from the diet.
- The pathogenesis of NCGS is not fully understood and may be different for individual patients but may be related to an innate immune response to gluten.

Abstract

Background There has been increasing interest in the entity of Non-Celiac Gluten Sensitivity (NCGS) in recent years; however, it still remains a controversial topic and its pathogenesis is not well understood. Celiac Disease, in contrast, is a well-studied condition that has become increasingly recognized as a prevalent condition arising from a heightened immunological response to gluten. Wheat allergy is an IgE-mediated condition capable of causing a variety of gastrointestinal symptoms. However, the number of patients who have neither celiac disease nor wheat allergy, but appear to derive benefit from a gluten-free diet, is also increasing substantially. The use of the term NCGS as a way of describing this condition has become increasingly prevalent in recent years.

Purpose In this review, we will focus on gastrointestinal manifestations of NCGS and discuss the evidence for the condition and its putative pathogenesis. We will discuss areas of controversy and areas for potential future research.

Keywords celiac disease, gluten sensitivity, irritable bowel syndrome, non-celiac gluten sensitivity.

INTRODUCTION

There has been increasing interest in the entity of Non-celiac gluten sensitivity (NCGS) in recent years; however, it still remains a controversial topic and its pathogenesis is not well understood. Celiac disease (CD), in contrast, is a well-studied, common condition resulting from the heightened immunological response to the ingestion of gluten-containing foods such as wheat, barley, and rye in genetically predisposed individuals.¹ The resulting inflammation leads to small-bowel villous atrophy. With the advent of improved serological testing, contemporary epidemiological studies have estimated the prevalence in the European adult population to be 1%.^{2,3} The gold standard diagnosis of

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CD is the demonstration of villous atrophy on duodenal biopsies, with celiac serology playing a supportive role.^{4,5} The cornerstone of treatment for CD is lifelong adherence to a strict gluten-free diet (GFD), which, in the majority, leads to an improved clinical outcome, psychological well-being, and quality of life.^{6,7}

A growing problem encountered in clinical practice is the diagnosis and management of patients complaining of gluten-related symptoms, yet, in the absence of diagnostic markers for CD, such as negative celiac serology and normal duodenal biopsies. Furthermore, an increasing observation noted is that the number of patients consuming a GFD seems greatly out of proportion to the projected number of CD patients. Although official data are lacking, the lay press have suggested that '17 million Americans are gluten sensitive'.^{8,9} However, it must be remembered that this is also 'big business', with Reuters projecting an increased revenue in the gluten-free market from \$1.31 billion (2011) to \$1.68 billion by the year 2015.¹⁰ These patients have frequently placed themselves on a GFD after negative experiences from eating gluten-containing foods and report significant improvements on a GFD.^{11,12} These patients can cause a diagnostic and therapeutic dilemma among gastroenterologists, general practitioners, and dietitians alike and in the past have been described as belonging to a 'no man's land'.¹³ In this review, we will appraise the current evidence for the diagnosis of NCGS and putative etiologies. We will discuss the uncertainties surrounding NCGS and highlight areas of future research that may elucidate this difficult clinical problem further.

WHAT IS NON-CELIAC GLUTEN SENSITIVITY?

In general, the definition of CD is fairly well understood by healthcare professionals and treatment is straightforward. However, in recent times, the clinical problem of patients who do not have CD, but complain of symptoms induced by wheat, has greatly increased. Other than case reports, a small case series in 1980 was the first to report a dramatic beneficial response to a GFD in nine of 17 patients experiencing abdominal discomfort and diarrhea related to the ingestion of gluten, but with no evidence of CD.¹⁴ Two decades later, a study of 94 adults affected by abdominal symptoms after cereal ingestion showed a prevalence of overt CD of 9%, 8% with latent CD, and 20% with cereal allergy. However, 63% of the study subjects could not be classified as CD or as allergic, but were yet affected by gluten-containing foods and clinically

benefited from a GFD.¹⁵ These were the first reports of patients with what is now defined as NCGS. This is widely recognized by the general public as a genuine issue; however, the scientific community has been slow to respond and there is a paucity of satisfactory clinical data surrounding this issue.⁹

Another of the difficulties in the understanding of gluten-related disorders is the often confusing nomenclature and variable definitions. Subsequently, this has led to the development of a consensus document, among a panel of 15 experts, on new classification of three-gluten induced and heterogeneous conditions: CD, wheat allergy, and NCGS. Wheat allergy is an IgE-mediated disorder, outside the scope of this review, leading to an array of symptoms of allergy from anaphylaxis to baker's asthma. Non-celiac gluten sensitivity was defined as a form of gluten intolerance that neither meets the diagnostic criteria for CD nor those for wheat allergy.¹⁶ Non-celiac gluten sensitivity is currently a diagnosis of exclusion with the only positive diagnostic criterion being the clinical response to gluten withdrawal. Patients should have negative celiac serology, normal duodenal histology, and negative IgE-based tests. Patients and physicians often find a diagnosis of exclusion less than satisfactory, particularly when many patients find a gluten-free diet unpalatable. As a result, there have been efforts to delineate positive criteria with a double-blind placebo-controlled challenge (DBPCC) used in a recent study of wheat sensitivity.¹⁷ However, DBPCC is currently only used as a research tool as it is time consuming and requires close follow-up of patients.

The prevalence of non-celiac gluten sensitivity was reported at 6% based on the Maryland secondary care clinic experience (where between 2004 and 2010, 5896 patients were seen with 347 fulfilling gluten sensitivity criteria).¹⁶ However, the true prevalence in the general population is unknown, particularly as many patients place themselves on a GFD without seeking medical attention. A recent American population-based survey suggested that the prevalence was 0.5%. However, the possibility of misclassification was a recognized methodological limitation.¹⁸

Non-celiac gluten sensitivity incorporates a wide range of intestinal symptoms including those consistent with irritable bowel syndrome (IBS), such as abdominal discomfort, bloating, pain, or diarrhea, or it may present with a variety of extra-intestinal symptoms, which may include headaches, foggy mind, depression, fatigue, musculoskeletal pains, and skin rash.^{12,19} For the purposes of this review, we will focus on the gastrointestinal (GI) manifestations of NCGS.

WHAT IS THE EVIDENCE THAT NCGS EXISTS?

As previously discussed, historical case series provided the only evidence of NCGS^{14,15} until two small unblinded studies were the first to show that a GFD may improve GI symptoms in a select group of patients with IBS.^{20,21} A more recent non-blinded randomized trial of 45 patients with diarrhea-predominant IBS-D, who had CD excluded by negative serology, a normal duodenal biopsy, showed a statistically significant improvement in stool frequency.²² However, this was a small mean difference from 2.6 to less than 2 bowel movements per day. The effect was more pronounced in patients with human leukocyte antigen (HLA) DQ2 or DQ8. Historically, our group has reported that gliadin antibodies were present in ~12% of the general population and ~17% in IBS, both in the presence of a normal small bowel biopsy.^{23,24}

The most robust evidence for a distinct clinical entity of NCGS comes from two recent double-blind placebo-controlled trials in patients with self-reported sensitivity to gluten²⁵ or wheat.¹⁷

The first study was a rechallenge trial study on 34 patients with IBS who were self-controlled on a GFD over a 6-week period. All patients had CD excluded based on negative celiac serology plus either a HLA genotype incompatible with CD or a normal duodenal biopsy. Patients were then subjected to either gluten or placebo in the form of muffins and bread that were low in fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAPs). Those in the gluten containing group demonstrated a significant deterioration of symptoms compared to those on a GFD. These symptoms included abdominal pain, bloating, satisfaction with stool consistency, and tiredness.²⁵ There was no difference between the groups in tests for intestinal injury in the form of fecal lactoferrin, C-Reactive Protein, and the dual sugar test for intestinal permeability. There was no change in celiac serology including gliadin antibodies on gluten challenge. The authors were therefore unable to elucidate a mechanism for the difference between the groups.

The second study was a larger study of 920 patients suffering from IBS.¹⁷ After a 4-week elimination diet, patients were rechallenged using a crossover DBPCC of wheat or placebo capsules. A total of 276 patients were identified as having wheat sensitivity, of whom 206 were identified as suffering from multiple food sensitivities. However, there is confusion in this terminology. There is still uncertainty as to whether it is gluten withdrawal that benefited patients or another

component of wheat. Contemporary reports suggest that fermentable fructans that are also present in wheat may provoke GI symptoms.^{10,26} Thus, the withdrawal of gluten may inadvertently be reducing the ingestion of other constituents of wheat, which interplay with gut microbiota, gas production, and fermentation.^{10,26,27} This is particularly pertinent to patients with IBS-type symptoms where recent interest and evidence to support the withdrawal of fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAPs) may also have an overlap with a GFD.^{28,29} Although patients enrolled in the study had CD excluded prior to entry, the criteria used for diagnosing CD may be another confounding factor. Patients had CD excluded on the basis of negative serology and a duodenal biopsy showing no evidence of villous atrophy. However, 94% of the patients with apparent non-celiac wheat sensitivity did have lymphocytic duodenitis (LD).¹⁷ Although LD is non-specific, it can be associated with CD,³⁰ particularly when associated with positive antiendomysium antibodies (EMA) in tissue culture^{31–33} as in 30% of the patients in this study. Patients with EMA in culture can go on to develop villous atrophy if they remain on a gluten-containing diet.^{34,35} It is possible therefore that some of the patients enrolled in the study may have had CD without villous atrophy, particularly as some patients had reduced wheat on enrollment in the study, which may have led to some improvement in histology.

These landmark studies have increased interest in NCGS; however, we must remain cautious in our diagnosis of NCGS. Indeed, recent evidence from a further double-blind placebo-controlled trial of patients with self-reported gluten sensitivity has thrown some doubt onto the diagnosis. Biesiekierski *et al.* used identical enrollment methods as in their 2011 study²⁵ to identify 37 patients with GI symptoms attributed to gluten.³⁶ Patients were then subjected to varying amounts of gluten for 7 days in an otherwise low-FODMAP diet. All patients in the trial benefited from a reduction in FODMAPs prior to the reintroduction of gluten or placebo. However, there was no difference in GI symptom scores between gluten and placebo arms. They demonstrated a significant nocebo effect with all groups demonstrating an increase in symptoms during the challenge period. No markers of intestinal injury were demonstrated in any of the groups. The reasons for the difference in these contradictory studies are not clear. Whether the placebo protein itself induced some symptoms or the crossover study design influenced the results needs clarification. These questions will only be answered with further well-designed placebo-controlled

studies to conclusively prove whether NCGS is a clinical entity distinct from FODMAP-induced symptoms and CD.

WHAT IS THE POTENTIAL PATHOGENESIS OF NCGS?

Elucidating a clear pathogenesis of NCGS is essential if we are going to be able to treat patients successfully, identify potential therapeutic targets, and fully understand the potential sequelae of the condition. Unlike CD, which has a fairly well-established pathogenesis, the cause of NCGS is not well understood. One of the main issues that has impeded progress is the difficulty in clearly defining patients groups to be studied. Several studies have used unselected patients with IBS-D as their test subjects rather than specifically patients with NCGS.^{22,37} There have been only three studies using a blinded placebo-controlled design and one of these studies used wheat rather than gluten as its intervention. There is also the possibility that patients with 'potential CD' or CD with lesser histological lesions may also have been included in some of the trials. All of these confounding factors make elucidating a clear pathogenesis difficult. However, areas of research have focused on two main areas.

Firstly, it has been hypothesized that symptoms may well be as a result of impaired intestinal mucosa barrier function. This would seem a sensible place to start as it is well established that CD patients have impaired intestinal permeability as a result of abnormal tight junction morphology.^{38–40} Tight junctions open in the presence of gluten and, in CD, this allows exposure of luminal antigens within the lamina propria.^{41,42} There are however conflicting data on whether intestinal permeability is affected in patients with NCGS. Biesiekierski *et al.* used a dual sugar absorption test to assess for changes in intestinal permeability and could not find any difference between the control and intervention arms of the study, although numbers were small and the test may not have been sufficiently sensitive to pick up small changes.²⁵ A study of 26 patients with NCGS, 42 patients with active CD, and 39 healthy control patients suffering with dyspepsia examined intestinal permeability using the lactulose to mannitol ratio (LAMA).⁴³ Patients with active CD had significantly increased intestinal permeability compared with healthy controls and NCGS. Non-celiac gluten sensitivity patients, on the other hand, had significantly reduced intestinal permeability compared with healthy controls. The characteristics of the tight junction proteins were also noted to be markedly different between those with CD and NCGS on real

time, quantitative polymerase chain reaction (PCR) analysis of small bowel biopsies. Claudins (CLDN) are protein components integral to the function of tight junctions. Patients with NCGS showed increased expression of *CLDN4*, compared with CD patients, which, along with *CLDN1*, is associated with normal tight junction function and intestinal permeability.⁴³ Data from a recent randomized controlled trial of 45 patients with IBS-D appear to be at odds with previous findings. Patients on a gluten-containing diet had significantly increased small intestinal permeability compared with those on a strict gluten-free diet.²² Patients in the gluten-containing arm of the study also showed reduced expression of *CLDN1*, occludin, and zonula occludins 1, all of which are associated with normally functioning tight junctions. The decrease in expression of these genes was also more pronounced in those with the HLA-DQ2/DQ8 haplotypes. So, what are the reasons for apparently contradictory data? The sample size of both studies is quite small and there are differences in the enrollment of patients. In both of the earlier studies, patients with a variety of symptoms induced by gluten were included with CD being ruled out following a gluten challenge,^{25,43} whereas, in the more recent study, patients were only included if they met criteria for IBS-D.²² Patients with impaired intestinal permeability may be expected to have diarrhea as a predominant symptom. Indeed, patients with post-infective IBS-D have been previously shown to have increased intestinal permeability.⁴⁴ Obviously, this does not however explain the apparent difference in intestinal permeability on exposure to gluten, but the lack of a healthy control arm makes interpretation of these results more difficult. It may be that as NCGS is a descriptive diagnosis covering a broad range of symptoms, it may include several different disease processes.

The immunology of NCGS is another interesting area of research that may elucidate a possible pathogenesis. It has been suggested that patients with NCGS show activation of innate immunity instead of adaptive immune mechanisms when compared with CD patients.⁴³ The exact immune process underpinning this is complex and, as yet, not fully understood. Gliadin has been shown to activate the release of interleukin (IL)-15 in both healthy individuals and those with CD.⁴⁵ Interleukin-15 is considered to be the first triggering signal of CD.⁴⁶ However, in healthy individuals, the IL-15 will be below the response threshold to elicit an inflammatory cascade, presumably due to decreased duodenal IL-15 receptor (IL-15R) expression. In patients with CD, an inflammatory response is triggered after the release of IL-15, as there

is an increased IL-15R expression coupled with a decreased immune response threshold against this cytokine⁴⁷—this subsequently leads to both an innate and a secondary adaptive immune response, ultimately manifesting as small intestinal damage. Patients with NCGS have been proposed to fulfill the criteria to stimulate an innate immune response, but seemingly fall short of triggering the adaptive immune response, presumably as they lack the associated machinery to do so, for example, the associated HLA typing or epithelial permeability. As a result, the inflammatory small bowel response is halted and mucosal manifestations are absent.⁴⁸ Recent work comparing mucosal expression in patients with CD and NCGS by Sapone *et al.* would support this hypothesis, as evidenced by significantly higher levels of adaptive immune markers [gamma-interferon (IFN- γ), IL-6, IL-21, and IL-17] in CD compared with NCGS. Conversely, the NCGS group had significantly higher expression of innate markers, such as the Toll-like receptor (TLR) 2, and, to a lesser extent, TLR 1.^{43,49} Biopsy specimens did however show an increase in CD³⁺ intraepithelial lymphocytes (IELs) when compared with controls. This was significantly less than CD patients, but it does give evidence of a limited adaptive immune response in these patients. Interestingly, a more recent study involving an *in vivo* gluten challenge of patients with CD and NCGS has shown evidence of an inflammatory response to gluten in NCGS patients.⁵⁰ Non-celiac gluten sensitivity patients showed an increased expression of IFN- γ compared with baseline. However, unlike NCGS patients, those with CD also demonstrated an increase in other adaptive pro-inflammatory cytokines tumor necrosis factor-alpha (TNF- α) and IL-8 after a 3-day gluten challenge. Again the density of CD³⁺ IELs in NCGS patients was also significantly higher than disease controls, but not as high as in CD patients and there was no significant change following gluten challenge.⁵⁰ This does seem to contradict previous evidence, but it must be noted that this trial was conducted solely in patients with the HLA-DQ2 haplotype, which is present in 95% of CD patients, but less than 50% of NCGS patients. It may be that patients with HLA-DQ2/DQ8 haplotypes with NCGS have a different pathogenesis compared with those without, or may in fact have CD, and results may therefore not be applicable to the whole NCGS population. A schematic of the potential immunology of gluten-related disorders is shown in Fig. 1.

Clearly, further work is required to fully understand the pathogenesis of this condition. Other avenues of research such as the effects of gliadin on intestinal transit and the makeup of intestinal flora in these

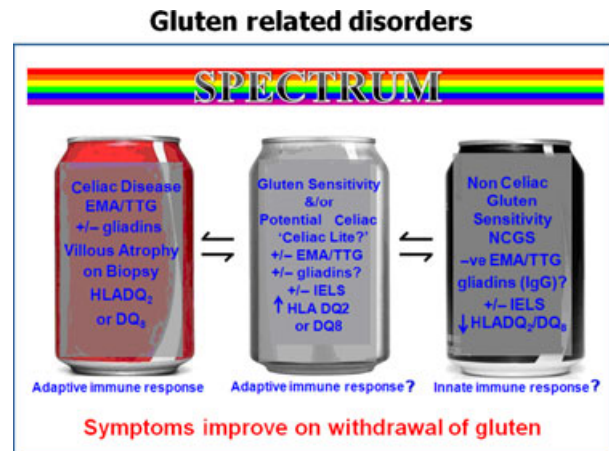


Figure 1 Spectrum of gluten-related disorders. All conditions have improvement on the withdrawal of gluten in common. Patients on the left hand side of the diagram have clear celiac disease with villous atrophy on biopsy. Patients in the other groups may be defined as having non-celiac gluten sensitivity, but they may have lymphocytic duodenitis or have the HLA phenotype associated with celiac disease. It may be that some of these patients may go on to develop overt celiac disease in the future. Patients on the far right of the equation show less in common with celiac patients and may be less likely to develop celiac disease. The pathophysiology in these patients may be due to an innate immune response. EMA, Endomysial Antibody; TTG, Tissue Transglutaminase; IEL, Intraepithelial Lymphocyte; HLA, Human Leukocyte Antigen.

patients warrant further investigation. It may be that, in future, further subdivision of patients into more clearly defined groups based on HLA typing, presence of gliadin antibodies, or symptoms may help clarify matters and aid successful diagnosis and management.

CLINICAL RELEVANCE AND FUTURE RESEARCH

Although recognition of NCGS has been around for some time, it is clear that there is a paucity of data in this field and also some skepticism surrounding the diagnosis of NCGS. Indeed, historically, many clinicians have been dismissive of symptoms and patients have frequently been referred for psychiatric assessments for their symptoms. However, recent evidence suggests that although patients with NCGS report more abdominal symptoms than CD patients, there is no evidence of abnormal personality traits or somatization to explain symptoms.⁵¹ From the recent double-blind placebo-controlled trials of exclusion diets, we now have evidence of abdominal symptoms caused by gluten or wheat in the absence of frank CD.^{17,25} Despite guidelines suggesting minimal investigation of patients presenting with typical symptoms of IBS,⁵² these patients frequently undergo multiple investiga-

tions often with negative results. Carroccio *et al.* have demonstrated a significant diagnostic yield in patients complaining of IBS type symptoms with a third of patients demonstrating improvement of symptoms on a wheat-free diet. Whether symptoms are caused by gluten alone or another component of wheat needs clarification. This does however validate the use of wheat exclusion in patients with IBS, although we do have to be aware of the possible nocebo effect of gluten/wheat ingestion.³⁶ Patients with IBS type symptoms frequently place themselves on highly restrictive diets and have been convinced of their effectiveness.⁹ Indeed, 14% of patients in Carroccio's study who did not have evidence of wheat sensitivity on DBPCC had self-reported symptoms attributed to wheat.¹⁷ In many patients therefore exclusion diets may not be appropriate and could have deleterious health consequences, but this can present a difficult diagnostic challenge with DBPCC being an unwieldy tool for day-to-day practice. It is important therefore that we look at ways of better classifying patients with NCGS. An accurate diagnostic test would certainly provide the best way of doing this. As previously discussed, gliadin antibodies and HLA-DQ2/DQ8 haplotypes are more prevalent in NCGS patients than the general population and it may be that they play a role in delineating NCGS patients in the future, but as yet they cannot be used in diagnosis. The flow cytometric basophil activation test or eosinophil counts in intestinal biopsies as used by Carroccio *et al.* may also prove to be useful diagnostic tools in the future, but further validation in different cohorts is required before such measures can be used on a clinical basis.^{17,53} Indeed, in a study of patients with IBS, Carroccio himself showed the variability in performance of different methods of the basophil activation test in food hypersensitivity.⁵⁴ The sensitivity of one commercially available basophil activation test was only

15–20% and specificity of 73% for cow's milk or gluten sensitivity confirmed by DBPCC.

CONCLUSIONS

Non-celiac gluten sensitivity currently provides the best description of a heterogeneous group of conditions that all have improvement in symptoms on the withdrawal of gluten in common. From a clinician's perspective, it is imperative that CD is ruled out in these patients. Thereafter, whether it is gluten withdrawal, wheat exclusion, or a low FODMAP that benefits the patient may be a process of trial and error. Whether the pathogenesis of this condition is the same for all patients remains to be seen and further work is clearly required to delineate patients more clearly. It is now clear that NCGS is a genuine clinical problem that patients at least have been aware of for years. As a scientific and clinical community, we now need to encourage more investment in research to aid diagnosis and identify potential therapeutic options. A dietary approach for the management of patients with gastrointestinal symptoms no longer seems elusive.

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