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# Controversies and challenges in non-celiac gluten/wheat sensitivity

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**Summary**

*Non-celiac gluten sensitivity (NCGS) has emerged as an intriguing and controversial topic in gastroenterology since the first reports over 40 years ago. The most recent definition requires a symptomatic reaction to gluten, or wheat containing food, the remission of symptoms with gluten or wheat challenge, and the exclusion of both celiac disease and wheat allergy. A definitive diagnosis of NCGS is challenging as there are no specific tests or biomarkers, and we still question the exact trigger for the condition. There have been several studies, including randomized-controlled trials (RCT), that aimed to understand whether it is gluten or the carbohydrate fraction in wheat, that trigger symptoms in non-celiac patients. Here, we review the literature to address outstanding controversies and challenges related to the diagnosis and management of this condition, as well as areas of interest for future studies.*

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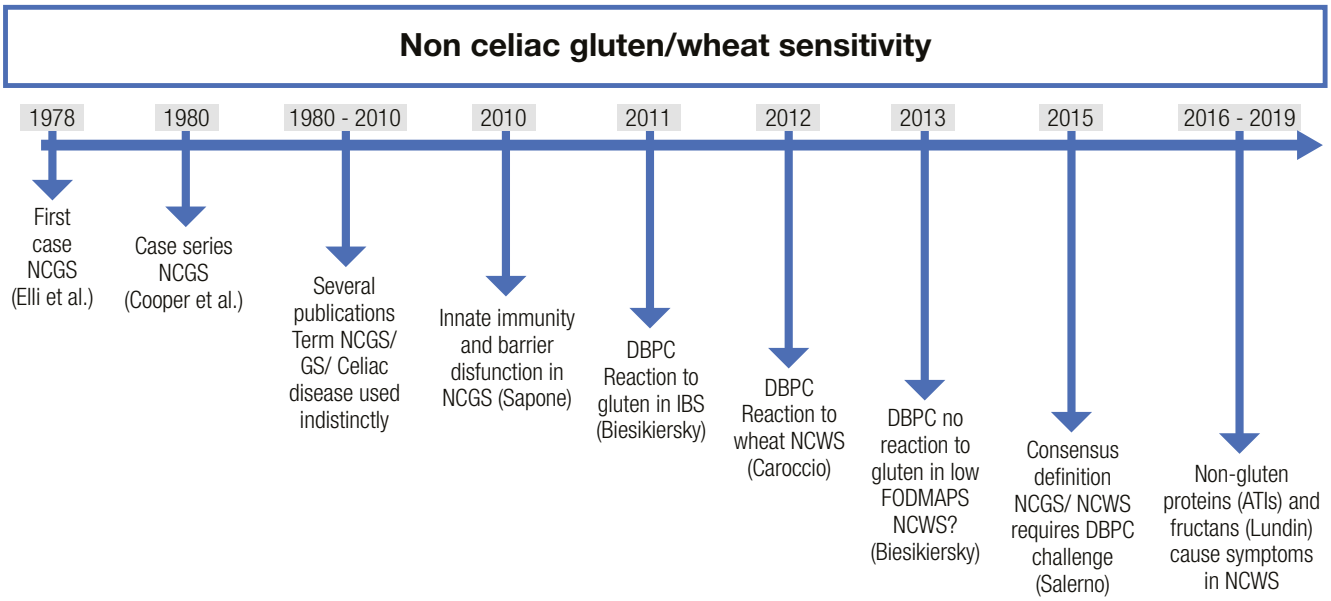
***Abbreviations***

*NCSG: non-celiac gluten sensitivity.*
*NCWS: non-celiac wheat sensitivity.*
*CD: celiac disease.*
*ATIs: amylase trypsin inhibitors.*
*FODMAPs: fermentable oligosaccharides, disaccharides, monosaccharides and polyols.*
*IBS: irritable bowel syndrome.*
*HLA: human leukocyte antigen.*
*TLR: toll like receptor.*

The term "non-celiac gluten sensitivity" (NCGS) was coined over 40 years ago, when Ellis and Linaker described the first case of diarrhea that improved with a gluten-free diet in the absence of celiac disease (CD).[5] However, the term "gluten sensitivity" has been frequently used as an umbrella to imply celiac disease, which has generated confusion. CD is defined as an autoimmune enteropathy induced by gluten in genetically susceptible people. It has a well understood pathophysiology and a recommended diagnostic work-up. Conversely, the debate regarding the definition and pathophysiology of NCGS endures, which has been recently renamed non-celiac "wheat" sensitivity (NCWS) (Figure 1).[2]

***Figura 1.*** *History and advances since the first report of non-celiac gluten sensitivity.*




It is important to realize the diagnosis of NCWS is performed almost entirely on the basis of clinical presentation and self-referral that gluten containing food induces symptoms. Diagnosis, therefore requires a symptomatic reaction to gluten, or wheat-containing food, in individuals in whom CD and wheat allergy has been ruled out.[3] The diagnosis is therefore based on the consensus definition of the condition, requiring (1) a symptomatic reaction to gluten/wheat, (2) symptom resolution after exclusion of wheat-containing food, and (3) re-appearance of symptoms with reintroduction of gluten/wheat products.[4] In the absence of validated biomarkers, a diagnosis of NCWS can only be made by a double-blind, placebo-controlled, dietary crossover challenge with gluten, which is difficult to apply in clinical practice.[5] Moreover, the challenge study does not confirm that the original symptoms, prior to challenge, were indeed due to gluten, carbohydrates or other components in wheat. Of people self-reporting gluten or wheat sensitivity, only a small proportion (16%) will have reproducible symptoms after a blinded gluten challenge of gluten versus placebo in a crossover dietary trial and fulfil the current consensus criteria for a diagnosis of NCWS.[5] This clearly highlights the difficulty and controversy in diagnosing and managing this condition. In this review we will focus on the most common challenges regarding NCWS, and the areas where research is needed.

## Controversies

"What's in a name?"

"A rose by any other name would smell as sweet" in William Shakespeare's play Romeo and Juliet, implies that the names of things do not affect what they really are. The term NCGS or NCWS has generated debate since the first reports.[1] The term "gluten sensitivity" implies immune reactivity to gluten, the immunogenic proteinaceous fraction in wheat that gives dough its consistency, and this has generated confusion with CD.[2] Thus, in the last years, there have been several consensus meetings attempting to re-define the condition.[6-8] To avoid confusion with CD, the Oslo consensus,[6] encouraged the use of the term NCGS instead of gluten sensitivity alone, and described one or more of a variety of immunological, morphological, or symptomatic manifestations precipitated by the ingestion of gluten in individuals in whom CD has been excluded. As defined by Sapone et al. NCGS is a reaction to gluten in which both allergic and autoimmune mechanisms have been ruled out, suggesting that NCGS is a diagnosis of exclusion.[7] The most recent consensus in Salerno, introduced a 2-step algorithm where patients should improve their symptoms in a 6 week period on gluten-free diet (GFD), followed by a second period of recurrence of symptoms after double blind placebo-controlled challenge for one

week with a crossover design.[3] The challenge should be performed with capsules containing 8 g of gluten, at least 0.3g of Amylase Trypsin Inhibitors (ATIs) in a Fermentable Oligosaccharides, Disaccharides, Monosaccharides and Polyols (FODMAPs)-free vehicle, and a gluten-free placebo.[3]

In a recent review, Catassi et al. introduced the new term "gluten or wheat sensitive IBS" which includes patients who present with irritable bowel syndrome (IBS)-type symptoms triggered by gluten.[8] In addition to IBS symptoms, patients may present a variety of gastrointestinal and extra-intestinal symptoms. Volta et al. reported that the most frequent extra-intestinal symptom was mental confusion or a "foggy mind", defined as a sensation of lethargy elicited by gluten, which is observed in 42% of the cases.[9, 10] Other extra intestinal symptoms identified were fatigue, skin rash, headache, joint and muscle pain (fibromyalgia-like syndrome), leg or arm numbness, depression, anxiety and anemia. Therefore, the clinical diagnosis of NCWS is not straight-forward and so far the most complex diagnosis proposed by the Salerno criteria is impractical. When patients with suspected NCWS are given a blinded gluten challenge vs. placebo as suggested by the Salerno criteria, only 22% will have confirmed NCWS.[11]

Other tests have been used to support the diagnosis of NCWS. For instance, duodenal biopsies performed in NCWS patients shows normal villous length, with or without increased intraepithelial lymphocytes and less likely increased eosinophils.[11, 12] While some have suggested immune reaction to gluten challenge, for example increased rates of basophils in biopsies of NCGS,[13] others were not able to confirm these findings.[14]

Unlike CD, there is no specific serology to diagnose NCWS. Anti-gliadin antibodies, which are not specific for CD, and can be present in many other gastrointestinal conditions, are found in about 50% of patients with suspected NCWS.[7, 9, 10] HLA-DQ2 and -DQ8 are present in 95-99% of CD patients, but carriage of these haplotypes in healthy people is common, being present in about 30-40% of most populations.[15] Some studies suggested that HLA-DQ2 and -DQ8 may be slightly more frequent in NCWS (~50%), however, this has been controverted by others.[15] In a recent study, Maki et al. evaluated genetic predisposition in 50 NCWS patients compared to non-NCWS controls.[16] They found that the most common genotype combinations within the gluten-sensitive cohort were A1-3/B2-6 and A1-5/B2-6, and the first was present only in NCWS population. Although the idea of identifying a genetic predisposition for this condition is exciting, it is not yet clear.

The lack of a clear clinical presentation and diagnostic tests to identify NCWS leads to difficulties in estimating properly its prevalence. In addition, the majority of patients that self-identify wheat as the responsible for their symptoms are already on a gluten-free or wheat-restricted diet by the time they consult a physician.[17] In a US study involving over 7000 people from the general population, Digiacomo et al.[18] identified NCWS in up to 6% of the US population, and the prevalence could be up to 13% if self-reported NCWS is considered.[17]

## Why do we need to understand the mechanisms involved in NCWS?

It is commonly accepted that understanding the mechanisms behind symptom generation is the key to develop research conducive to a treatment or clinical management. Complex disease is difficult to manage because it is often multifactorial. The potential mechanisms involved in the pathophysiology of NCWS are many, and include, as in IBS, alterations in intestinal permeability, stimulation of innate immunity and changes in gastrointestinal motility. The main distinction between IBS and NCWS would be the trigger: While IBS has many triggers, gut dysfunction and symptom generation in NCWS, is assumed to be induced mainly by wheat consumption. As such, NCWS could be considered part of the spectrum of food-induced IBS. In terms of clinical picture, others argue that NCWS presents with a plethora of non-digestive symptoms (fatigue, etc.) that, although also present in some IBS patients, does not clinically define the syndrome, whose diagnosis relies on abdominal pain and change in bowel function.[9, 10] Therefore, it could be hypothesized that specific mechanisms apply to NCWS that differ from those in general IBS.

There has been particular interest in clarifying whether intestinal permeability is altered in NCWS. An initial study by Sapone et al. found no alterations in intestinal permeability in NCWS compared with CD.[19] Subsequent studies found opposite results, which may be related to differences in methodology between studies, and more importantly, variances in defining NCWS which may lead to selection bias.[20-23]

Immune tolerance to dietary antigens is the key to prevent undesirable responses to innocuous antigens ingested with food; therefore, loss of tolerance to gluten or other wheat proteins in NCWS could involve an

immune mediated mechanism.[24, 25] In vitro studies have demonstrated that digests of gliadin increase the expression of co-stimulatory molecules and the production of proinflammatory cytokines in monocytes and dendritic cells.[26, 27] In addition, clinical studies have demonstrated increased expression of TLR-2 in the intestinal mucosa of non-celiac compared to celiac patients, suggesting a role of the innate immune system in the pathogenesis of non-celiac reactions to gluten or other wheat components.[19] In a recent study, Gomez Castro et al. have shown that the p31-43 peptide (p31-43) from α-gliadin can induce an innate immune response in the intestine and that this may initiate pathological adaptive immunity.[28] The role of this or other immunogenic peptides in NCGS/WS is not clear, and yet needs to be explored.

It is important to stress that other proteins in wheat, such as α-amylase/trypsin inhibitors (ATI) and wheat lectin agglutinin, have recently shown to induce innate immune pathways[2] and has opened the field to other non-gluten trigger proteins in wheat as responsible of symptoms in non-celiac population. There is an increasing interest in the role of ATI as activators of innate immune responses in monocytes, macrophages and dendritic cells through toll like receptor (TLR) 4-MD2-CD14 complex.[29] Furthermore, a recent study by Caminero et al. has shown greater immune activation in mice fed both gluten and ATI compared to those fed only gluten or only ATI, indicating a potential synergistic effect.[30]

In addition to protein fractions, wheat contains a group of carbohydrates, called fructans, which are members of fermentable oligosaccharide, disaccharide, mono-saccharides, and polyols (FODMAPs) that are poorly absorbed in the small intestine.[2] In a placebo-controlled, cross-over rechallenge study Biesikiersky et al. suggested that gluten had no effect in patients with self-reported NCGS after dietary reduction of FODMAPs, however, this study included a mixed population of IBS patients that likely respond to a myriad of dietary exclusion diets, including placebo.[31] Other methodological issues such as short wash-out periods between challenges may difficult the interpretation of results. A recent study by Skodje et al. suggested that fructans rather than gluten, induces symptoms in self-reported NCWS. The mechanisms for these reactions were not investigated in any of this studies.[32]

Changes in gastrointestinal motility and gut micro-biota have been proposed as potential mechanism in NCWS. Animal studies have shown that gliadin can trigger smooth muscle hyper-contractility and cholinergic nerve dysfunction in mice expressing the human leukocyte antigen (HLA)-DQ8 genes, but without evident duodenal atrophy.[27]

Few clinical studies have investigated changes in gastrointestinal motility triggered by wheat components. Even though an initial study by Vázquez Roque[21] showed that gluten-free diet had no significant effects on gastrointestinal transit in a cohort of IBS patients with diarrhea, a more recent study by our group showed that, normalization of gastrointestinal motility in IBS patients was limited to those with anti-gliadin antibodies.[33]

## The search for trigger/s in NCWS is embroiled in polemics

One of the most controversial areas in NCWS is identifying whether gluten or other wheat protein or carbohydrate fractions trigger symptoms.

Several clinical trials (Table 1) as well as mouse studies, have attempted to address this issue with controversial findings. Gluten has been an obvious target since the condition was first described.[1] Different randomized placebo controlled trials[34-40] has shown that a gluten challenge triggers gastrointestinal and extra-intestinal symptoms.

The main limitation is that gluten challenge is rarely a pure challenge and as previously noted, wheat contains other immunogenic proteins[29, 30] and carbohydrates,[31, 32] that may contribute to symptoms in NCWS alone or through a synergic effect. However, the evidence in clinical setting is scarce.

The effect of a diet low in ATI has not yet been tested in a clinical trial, and there is only one trial evaluating the role of fructans in patients with NCWS or IBS. Finally, it remains unclear why some people who are perfectly healthy suddenly experience NCWS. Although the predisposing factors are unknown, it has been suggested that dysbiosis following a gastroenteritis may lead to the development of NCWS.[41]

There are different mechanisms by which microorganisms might provide direct pro-inflammatory signals to the host promoting breakdown of oral tolerance to food antigens or indirect pathways that involve the metabolism of protein antigens and other dietary components by gut microorganisms, which may explain reactions to wheat.[42]

***Table 1.*** *Summary of clinical trials in NCGS/NCWS (Adapted from Pinto-Sanchez et al., NMO 2018).*

| Author (year) | Design | n | Challenge | Dose | Control | Treatment | Wash-out | Run-in | Outcome |
|---|---|---|---|---|---|---|---|---|---|
| Biesiekierski 2011[34] | DB-RCT | 34 | Muffins/bread slices | 16g gluten/day | GF muffin or bread | 6 weeks | - | - | Gluten triggers IBS symptoms |
| Caroccio 2012[48] | DBPC | 50 | Capsules wheat | 10 g/day | xylose | 2 weeks | 1 wk | 4 weeks elimination diet | Wheat triggers symptoms |
| Biesiekierski 2013[31] | DB cross-over | 22 | Mals high in gluten doses | 16 g/day | Low gluten 2 g/day or placebo (whey protein 16 g) | 2 weeks | 2 wks | 6 weeks GFD and 2 weeks low FODMAP | Gluten did not trigger symptoms in low FODMAP |
| Di Sabatino 2015[35] | DBPC cross-over | 61 | Capsules gluten | 4.375 g /day | Placebo-rice starch | 1 week | 1 wk | GFD | Gluten triggers IBS symptoms |
| Shahbazkhani 2015[36] | DBPC | 72 | Sachet powder gluten meal low FODMAP | 50 g/day | Sachet powder rice flour | 6 weeks | - | GFD 6 mo | Gluten triggers IBS symptoms |
| Zanini 2015[38] | DBPC cross-over | 35 | Sachet powder vital gluten | 10 g/day | Sachet gluten free flour | 10 days | 2 wks | GFD > 3 mo | Gluten triggers IBS symptoms in a minority |
| Elli 2016[39] | DBPC cross-over | 98 | Capsules gluten | 5.6 g/day | Rice starch | 7 days | 7 days | GFD x 3 weeks | Gluten triggers IBS symptoms in a minority |
| Zanwar 2016[37] | DBPC | 60 | Bread | - | GF bread | 4 weeks | - | GFD 1 mo | Gluten triggers IBS symptoms |
| Skodje 2017[48] | Open challenge | 56 | Bread | 120 g bread | No control | 3-14 days | - | GFD 16 mo | Wheat triggers symptoms |
| Skodje 2018[32] | DBPC cross-over | 59 | Muesli bars gluten + FOS | 5.7 g gluten 2.1g FOS | GF low FODMAP muesli bar | 7 days | 7 days | GFD > 6 mo | Fructan triggers IBS symptoms |
| Dale 2018[40] | DBPC | 20 | Muffin gluten | 11 g pure gluten | Rice starch | 4 days | 3 days | GFD 6 weeks | Gluten does not trigger symptoms in most IBS |
| Haro 2018[45] | Open challenge | 10 | Low gluten bread | 100 g bread (< 1 g) | GF bread | 7 days | No | No | Tolerance small quantities of gluten. Beneficial changes microbiota |
| Roncoroni 2019[44] | Open challenge | 22 | Incremental gluten containing diet | Diet with low (3.5 g/ day) | Mod (8 g/d) High (13 g/d) gluten | 7 days | No | No | Dissimilar responses to different doses of gluten |

**DB-RCT:** double-blind randomized controlled trial; **PC:** placebo controlled; **GFD:** gluten-free diet.

## How do we optimally manage a condition that is difficult to diagnose?

The gluten-free diet has become increasingly popular around the world, and the overall interest in gluten-free diet has been growing from 2004 to 2019; which is beyond the interest in CD (Figure 2). This implies the majority of people interested in a gluten-free diet, may not necessarily have CD. Not surprisingly, there has been a well-known rapid market growth of gluten-free foods, due to an alarming number of individuals voluntarily adopting a gluten-free or restricted diet.[24]

The only available therapy for NCWS is avoidance of wheat products; however, the long-term health consequences of this are unknown. Gluten-free diet has been associated with nutritional deficiencies which are independently of the condition,[24] which may raise some awareness at the time of prescribing this restrictive diet. In addition, potential nutritional deficiencies, wheat restriction has been shown to affect the richness and composition of small intestinal and fecal microbiota, reducing bacterial groups actively involved in carbohydrate fermentation such as Firmicutes.[23, 43]

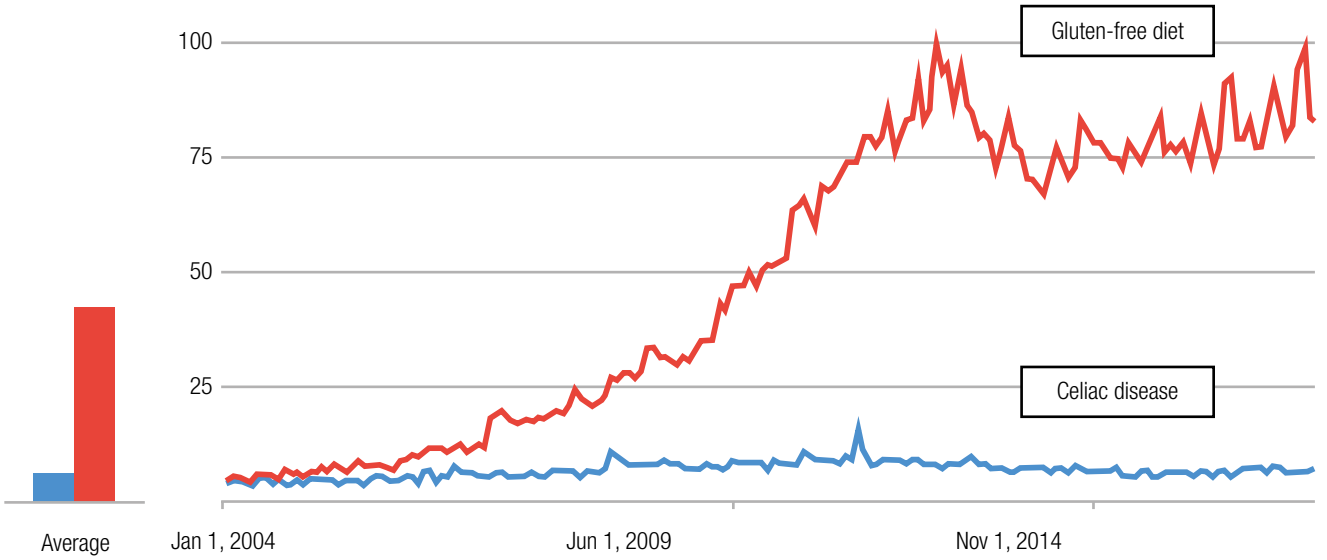
Unfortunately, the previous concerns will also apply to other restrictive diets such as the low in FODMAPs. Therefore, deciding whether to start or not a gluten-free diet in a patient with suspected NCWS is a real concern. To complicate things further, not all patients with NCWS will respond to a wheat restricted diet. As suggested by three recent exploratory studies evaluating symptoms reaction to different doses of gluten in the diet, gluten/wheat restriction rather than gluten/wheat free diet may be sufficient in NCWS population.[32, 44, 45] If a decision to trial a gluten-free diet is taken, the most important clinical step is to rule out CD, with specific serology and endoscopy in that patient, prior to starting the restrictive diet. (Table 2).

## Future research in NCWS

The definition of non-celiac gluten or wheat sensitivity has changed over the years and there is still no consensus on the most appropriate term to define the condition.

There is more than one symptom in NCWS, more than one potential component in wheat capable of triggering dysfunction, and hence the answer is likely to be complex and involve more than one trigger. However, it will be crucial to identify what the key wheat components are, alone or in combination. This is the only way we will advance in better therapeutic approaches aimed at selectively reducing those components in wheat-derived

***Figura 2.*** *There is more interest over time in the gluten free diet than in celiac disease.*




https://trends.google.com/trends/explore

products. As opposed to CD where gluten content in food should be kept below 20 ppm/day, no such threshold has been identified in NCWS and it may be sufficient to reduce the quantity of some wheat proteins or fructans to a level that is tolerable to these patients. Adequate randomized-controlled trials with crossover design and appropriate washout period, comparing different components are strongly needed to identify this. In addition to multiple triggers, it is possible that, as in IBS, subgroups of people have different susceptibility to different wheat components.

Therefore, we should continue the search for biomarkers that may help identify subgroups of patients who may benefit from particular dietary counselling.

Recommendations on whether, or not, to start a restriction diet should be based on the evidence that benefits outweigh the associated risks. Additional therapies such as probiotics[46] or bacteria derived enzymes[47] have been proposed as adjuvant treatment for NCWS, however, the evidence is scarce to date, which highlights the need of research in this area.

***Table 2.*** *Controversies and challenges in NCGS/NCWS.*

| | Controversies | Challenges | Future research |
|---|---|---|---|
| **Diagnosis** | - Clinical presentation: IBS and/or extra-intestinal symptoms<br>- Need of DBPC challenge<br>- Antigliadin antibodies are present in the majority of NCGS/NCGS<br>- IELs and basophils may be increased in biopsies | - Differentiate NCGS from IBS<br>- DBPC challenge is Impractical<br>- AGAs are non-specific<br>- Normal biopsies are found in 50% NCGS/NCWG | Discovery of biomarkers that predict response to GFD in non-celiac population |
| **Prevalence** | - Prevalence of NCGS is 0.6-6% | - Lack of biomarker to diagnose NCGS/WS | Epidemiological studies to estimate the true prevalence of NCGS |
| **Triggers** | - Gluten or other wheat components such as ATIs, fructans induce symptoms<br>- Dysbiosis may trigger symptoms | - There may be more than one wheat component to trigger symptoms, and also, different components may trigger symptoms in different subgroups of NCGS/WS | RCT DBPC cross over design are needed to identify which specific wheat component or combination of them induce symptoms in NCGS/NCWS |
| **Mechanisms** | - Innate immunity, permeability, changes in GI motility, changes in microbial diversity may contribute to the pathophysiology | - Studies on mechanisms are challenging in clinical setting; studies in mice are needed | Preclinical studies to identify immune markers, and clinical studies evaluating whether changes in motility, and microbial diversity may explain symptoms |
| **Treatment/ Management** | - Need of strict GFD<br>- Need of assessment of GFD compliance in NCGS/NCWS<br>- Probiotics may be beneficial in NCGS/WS<br>- Microbial derived enzymes may benefit NCGS/WS | - Confirmation that symptoms are triggered by gluten/wheat<br>- Identifying which probiotic/mix of probiotics will benefit | Determine the level of gluten tolerance, and other adjuvant therapies (such as probiotics or microbial derived enzymes) to induce tolerance |

**DBPC:** double-blind placebo controlled; **IELs:** intraepithelial lymphocytes; **AGAs:** antigliadins.

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***Competing interests.*** *The authors have no competing interests to declare.*

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