The spectrum of noncoeliac gluten sensitivity

Imran Aziz, Marios Hadjivassiliou and David S. Sanders

Abstract | The past 5 years have seen an increase in the use of a gluten-free diet outside a diagnosis of coeliac disease or IgE-mediated wheat allergy. This trend has led to the identification of a new clinical entity termed noncoeliac gluten sensitivity (NCGS). In this Review, we discuss the evidence for NCGS as demonstrated by the results of double-blind, placebo-controlled dietary rechallenge studies. Furthermore, the characteristic phenotype of individuals with NCGS is described as well as the symptom manifestations commonly reported after gluten exposure, which include intestinal symptoms consistent with IBS, and extraintestinal symptoms such as neurological dysfunction, psychological disturbances, fibromyalgia and skin rash. Moreover, emerging evidence suggests that NCGS can be associated with organic gastrointestinal pathologies, such as IBD, in which its presence might be a reflection of severe or stricturing disease. However, NCGS is not without its controversies and uncertainties, in particular pertaining to whether it is gluten or nongluten components of the grain evoking symptoms; evidence suggests that fermentable carbohydrates, amylase trypsin inhibitors and wheat-germ agglutinin can also be responsible culprits. Finally, we discuss the novel techniques that might help diagnose NCGS in the future.

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Introduction

The Neolithic revolution saw a transition from hunting and gathering of food to settled agriculture. The first signs of wheat cultivation can be attributed to the Fertile Crescent in South Western Asia dating from ~9000 BC to 4000 BC. The popularity of wheat as a staple food was quickly established owing to its high nutritional value and palatability when processed into products such as bread, pasta, couscous and beer. Since that time, wheat has become among the most grown crop worldwide with modern day production amounting to >700 million tonnes per year. To enable such an efficient agricultural system, wheat strains have had to undergo selective breeding whereby those with best adaptation to extreme climate conditions, bread-making qualities and resistance to diseases can be selected. This change in genetic variety and immunogenic qualities of wheat might have led to the development of gluten-related disorders.[1]

Coeliac disease was the first gluten-related disorder to be described. The condition affects 1% of the population and is defined as a small intestinal immune-mediated enteropathy precipitated by exposure to dietary gluten in genetically susceptible individuals.[2] All patients with coeliac disease carry the HLA-DQ2 and/or HLA-DQ8 genotypes, although these alleles are present in ~40% of the general population.[3] Individuals with coeliac disease can present with either the classic symptoms of malabsorption, such as diarrhoea and weight loss, or with nonclassic symptoms that include IBS-type symptoms, haematinic deficiencies and fatigue.[2] The diagnosis of coeliac disease is based on the demonstration of histological abnormalities on duodenal biopsies, ranging from increased density of intraepithelial lymphocytes (IELs, >25 per 100 enterocytes) to villous atrophy, in the presence of positive serology for coeliac-disease-specific antibodies. In the past, serum antigliadin antibodies (AGAs) were used, but in view of their poor diagnostic accuracy for the presence of enteropathy they have been superseded by the highly sensitive and specific anti-endomysial antibodies and anti-transglutaminase 2 (TG2, also known as tissue transglutaminase) antibodies.[4,5] Once a diagnosis of coeliac disease is made, the cornerstone of treatment is lifelong adherence to a strict gluten-free diet (GFD), leading to clinical and histological remission.

Historically, individuals with coeliac disease have struggled in managing a GFD with adherence ranging from 42–91%.[6] This variable adherence can be explained partly by gluten-free products being more expensive and having limited availability, as well as the general public and chefs lacking knowledge regarding coeliac disease or a GFD.[7–9] These factors have contributed towards social phobia and fear of dining out in individuals with coeliac disease.[10] However, the past 5 years have seen a dramatic shift in the availability of gluten-free products in tandem with increased societal awareness.[11] This trend is mainly due to the sudden increase in individuals without a formal diagnosis of coeliac disease self-prescribing a GFD. The media have been at the forefront in recognizing the growing popularity of a GFD, estimating that 15–25% of US consumers want gluten-free foods and that by 2017 the market will be worth US$6.6 billion dollars.[12,13] Over the past few years, several population-based observational studies have confirmed the avoidance of gluten-based products outside a diagnosis of coeliac

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Key points

- Individuals are increasingly self-reporting gluten sensitivity and placing themselves on a gluten-free diet outside a diagnosis of coeliac disease or IgE-mediated wheat allergy
- This clinical entity has been termed noncoeliac gluten sensitivity (NCGS)
- The symptoms evoked by gluten in NCGS include a constellation of intestinal and extraintestinal symptoms
- Nongluten components of the grain can also be responsible for triggering symptoms in individuals with NCGS
- No diagnostic biomarkers to differentiate between gluten and nongluten components currently exist; positive antigliadin antibodies support the diagnosis of NCGS but have limited sensitivity and specificity
- Patients presenting with NCGS are a heterogeneous group and should be counselled about the uncertainties surrounding their diagnosis

disease (Table 1).[14–19] The reasons for adopting such a lifestyle can be varied; in some it is perceived as a healthier option and a means of controlling weight gain by reducing calorific intake.[18] However, most individuals taking a self-prescribed GFD do so with the view that gluten exposure triggers symptoms of ill health.[18]

The first clinical cases published on individuals with gluten sensitivity in the absence of coeliac disease date back to the mid-1970s.[20,21] These brief reports describe a small number of young to middle-aged women presenting with a longstanding and previously unresolved history of abdominal pain, discomfort, bloating, altered bowel habit and fatigue. Extensive gastrointestinal investigations were all negative, including the exclusion of coeliac disease. Their symptoms would seem compatible with the criteria used to diagnose IBS, although the physicians did not state this diagnosis in their report. With various treatment options failing, an empirical trial of a GFD led to a remarkable improvement in clinical symptoms with subsequent relapse on gluten challenge.[20,21] One of these groups described how in six patients, now well controlled on a GFD, double-blinded crossover exposure to gluten-containing flour versus gluten-free flour led to notable symptom induction in the group on gluten.[22] However, whether participants were able to differentiate between the two challenges based on taste and texture, particularly as the gluten-free flour was commercially available and commonly prescribed for the coeliac diet, is unclear. Despite the promising findings, this study proved to be controversial and was met with some scepticism.[23,24] It is difficult to know how such individuals were perceived by their family practitioners or gastroenterologists, but given the considerable paucity of further publications in the field for the next 30 years it has been suggested that they might have been left in a 'no-man's land' and potentially dismissed as having an underlying psychosomatic ailment accounting for what appeared to be nonsensical gluten-related symptoms.[25] However, with mounting media speculations regarding the gluten-free lifestyle, further clinical studies have now been performed to elucidate the nature of this relationship, leading to the identification of a new clinical entity termed noncoeliac gluten sensitivity (NCGS). In this Review, we discuss the evidence for NCGS and its surrounding controversies and uncertainties.

The evidence for IBS and NCGS

IBS is common with a pooled global prevalence of 11.2%.[26] The aetiology of IBS is unclear, but in one study 84% of patients with IBS believed that food items were important triggers of their gastrointestinal symptoms, with gluten-based products causing symptoms in 24% of patients.[27] Furthermore, patients with IBS who report adverse food reactions tend to have more severe symptoms, associated subjective health complaints of musculoskeletal pains and chronic fatigue, and reduced quality of life compared with patients with IBS without food sensitivities.[27–29] These adverse reactions can be due to an allergy, intolerance or sensitivity. In accordance with the Rome Foundation Working Group, an allergy implies a specific immune response (IgE-mediated or non-IgE-mediated) that occurs reproducibly on exposure to a particular food component.[30] An intolerance or sensitivity has no established immune-mediated reaction.[30] Whereas IgE-mediated food allergy can be detected with the use of skin prick test and specific IgE-based serology, confirming non-IgE-mediated food allergy or food sensitivities can be

Table 1 | Observational studies assessing the use of a GFD and known diagnosis of coeliac disease

Author	Country	Group	Sample size	Avoidance of gluten-based products	Known previous diagnosis of coeliac disease
Tanpowpong *et al.* (2012)[14]	New Zealand	Children, general population	916	5.24% (n=48)	0.98% (n=9)
Rubio-Tapia *et al.* (2012)[15]	USA	Age ≥6 years, NHANES 2009–2010	7,798	0.63% (n=55)	0.08% (n=6)
Aziz *et al.* (2014)[16]	UK	Adults, general population	1,002	3.69% (n=37)	0.80% (n=8)
Lis *et al.* (2015)[17]	Australia	Adults, athletes	910	41.20% (n=375)	None
Golley *et al.* (2015)[18]	Australia	Adults, general population	1,184	10.64% (n=126)	1.18% (n=14)
Mardini *et al.* (2015)[19]	USA	Age ≥6 years, NHANES 2009–2010 and 2011–2012 data combined	14,701	0.97% (n=142)	0.14% (n=21)

Abbreviations: GFD, gluten-free diet; NHANES, National Health and Nutrition Examination Survey.

difficult in routine clinical practice owing to the absence of biomarkers and the cumbersome, time-consuming nature of performing the gold-standard method, which is dietary elimination followed by double-blind, placebo-controlled (DBPC) food rechallenges.[31]

In this regard, coeliac disease can be considered a non-IgE-mediated allergy (along with its associated autoimmunity), which tends to give rise to symptoms days to weeks after gluten exposure. By contrast, IgE-mediated wheat allergy usually manifests immediately, within minutes to hours, and can affect the skin, respiratory, or gastrointestinal tract. However, studies have shown that gluten-based products can also cause gastrointestinal symptoms, usually within hours to days after exposure, in the absence of coeliac disease or IgE-mediated wheat allergy.[32,33] Carroccio *et al.*[32] recruited 920 adults with IBS who self-reported gluten-based sensitivity without evidence of coeliac disease or IgE-mediated wheat allergy. After 4 weeks of a dietary elimination period, patients underwent a DBPC challenge of receiving either wheat or xylose capsules for 2 weeks then a 1-week washout before receiving the other capsule for another 2 weeks. The investigators found that 30% reacted to the wheat challenge, which induced symptoms of abdominal pain, bloating and altered stool consistency. Two distinct groups were identified: those with wheat sensitivity alone and those with wheat sensitivity associated with multiple food sensitivities.[32,34] Despite these novel findings, the main limitation of this study is that wheat was used, making it impossible to differentiate whether it was gluten or a nongluten constituent in wheat-evoking symptoms.

Biesiekierski *et al.*[33] recruited 34 patients with IBS who self-reported gluten sensitivity. The investigators ensured adequate exclusion of coeliac disease as demonstrated by negative HLA-DQ2 and HLA-DQ8 genotypes, or normal duodenal biopsies on a gluten-containing diet in those individuals expressing the HLA-DQ2 and/or HLA-DQ8 genotypes. Thereafter, participants were symptomatically controlled on a GFD and underwent a DBPC challenge of receiving snacks of either 16 g of gluten per day or placebo for up to 6 weeks. The snacks were free of fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAPs). Subsequent visual analogue scale ratings revealed that within the gluten group, 68% reported that their symptoms were inadequately controlled compared with 40% in the placebo group ($P = 0.0001$). Within 1 week, overall symptoms, abdominal pain, bloating, stool dissatisfaction and tiredness were substantially worse with gluten.

In 2012, experts in gluten-related disorders produced a consensus document in which a new clinical entity termed NCGS was introduced.[35] Owing to the absence of diagnostic biomarkers, NCGS has been defined as gluten-related symptoms without evidence of coeliac disease or IgE-mediated wheat allergy.[35,36] However, after the publication of this consensus document, the existence of NCGS was called into question by a subsequent study showing no specific or dose-dependent effects of gluten and instead implicating that nongluten components, specifically FODMAPs, might be responsible symptom triggers.[37]

Box 1 | Characteristic phenotypes of self-reported NCGS*

Female prevalence: 72–84%
Mean age: 38 years

Lower gastrointestinal symptoms
- Diarrhoea: 16–54%
- Constipation: 18–24%
- Altered bowel habit: 27%
- Abdominal pain/discomfort: 67–83%
- Bloating: 72–87%
- Weight loss: 25%

Upper gastrointestinal symptoms
- Epigastric pain: 52%
- Nausea: 9–44%
- Aerophagia: 36%
- Gastro-oesophageal reflux: 32%
- Aphthous stomatitis: 31%

Extraintestinal symptoms
- Skin rash (eczema or dermatitis): 6–40%
- Brain: depression 15–22%; foggy mind 34–42%; anxiety 39%; confusion 5%; headaches 22–54%
- Limb numbness: 6–32%
- Joint or muscle pains (fibromyalgia-like symptoms): 8–31%
- Fatigue: 23–64%
- Lack of well-being: 68%

Data taken from several reports.[16,35,39,40] *In adults. Abbreviation: NCGS, noncoeliac gluten sensitivity.

The clinical phenotype of NCGS

Studies evaluating NCGS have generally been performed in adults, although paediatric cases have been reported.[38] A prospective multicentre Italian survey performed over 1 year identified 391 new cases of NCGS to 340 new cases of coeliac disease, giving a ratio of 1.15:1.[39] In this cohort, the breakdown for adults was 380 NCGS to 302 coeliac disease cases (ratio of 1.25:1), whereas for children there were 11 NCGS to 38 coeliac disease cases (ratio of 0.29:1). These findings support the concept that NCGS is primarily seen in adults.[39]

The characteristic phenotype of patients with suspected NCGS is usually young to middle-aged women describing a constellation of both intestinal and extraintestinal symptoms after gluten exposure (Box 1).[16,35,39,40] In most patients, the time between gluten ingestion and the appearance of symptoms varies from a few hours to 1 day.[39] The most frequent associated disorders are IBS (48%) and other food intolerances (35%), which in most cases are represented by lactose intolerance, and IgE-mediated allergy (22%) to inhalants, food, or metals.[39] When comparing baseline parameters, patients with NCGS are generally more likely to have nutritional deficiencies, coexisting autoimmunity, a lower mean BMI and decreased bone mineral density than the general population; however, these complications are seen less frequently in NCGS than in coeliac disease.[16,40–42] The prevalence of a family history of coeliac disease can be seen in 5–24% of patients with NCGS.[16,32,39–43] This finding might reflect that individuals who self-prescribe a GFD do so because they are aware of coeliac disease, and its protean manifestations, through their family history. However, up to half of siblings of patients with coeliac disease who do not have

the condition demonstrated gluten sensitivity following a rectal gluten challenge,[44] suggesting that within a family various degrees of gluten sensitization exist that requires further exploration. The prevalence of HLA-DQ2 and/or HLA-DQ8 genotypes is ~50% in NCGS, which is substantially less than in coeliac disease but comparable to the general population.[16,40] Although no biomarkers have been identified to date, 25–50% of patients with NCGS can have serum AGA, mainly IgG class.[32,39,40,45–47] However, AGAs lack specificity as they can be present in the general population and healthy blood donors (2–12%), and in patients with IBS (6–17%), connective tissue disorders (9%) or autoimmune liver diseases (21.5%).[48] Nevertheless, their presence in the context of NCGS would help support its diagnosis, as a GFD correlates with clinical and serological remission.[45] Duodenal biopsies in NCGS are normal or demonstrate a mild increase in IEL numbers, usually ranging from 25–40 per 100 enterocytes, which is less than that characteristically seen in coeliac disease.[46,47] Furthermore, the duodenal IEL pattern in NCGS can have a peculiar distribution, with clusters of lymphocytes in the superficial epithelium and linear deposition within the lower portion of the lamina propria.[49,50] However, these findings have also been seen in IBS cases without NCGS, indicating a lack of specificity.[50]

The immunopathogenesis of NCGS

The immunopathophysiology underpinning NCGS is largely uncertain, with discordant data.[51] In one study, the mucosal response to gluten exposure differed between NCGS and coeliac disease.[46,47] Whereas gluten triggered only an innate immune response in NCGS (as demonstrated by increased expression of Toll-like receptors), and showed reduced intestinal permeability with increased expression of tight junction protein claudin-4, it provoked an additional adaptive immune response (increased expression of IFN-γ, IL-6, IL-21,and IL-17) plus increased epithelial permeability in coeliac disease.[46,47] However, increased expression of IFN-γ has been shown in NCGS, opening the possibility of an adaptive component;[52] this concept can be supported by the synthesis of AGAs, seen in a proportion of patients with NCGS, and which can be viewed as activation of adaptive immunity. Elsewhere, preliminary studies on NCGS have demonstrated decreased expression of tight junction proteins in both the small bowel and rectosigmoid mucosa, reduced intestinal barrier function, increased small bowel intestinal permeability, proliferation of peripheral blood monocytes, flow cytometric basophil activation in *in vitro* assays, and eosinophil infiltration of the duodenal and colonic mucosa.[32,53,54] These findings might present a plausible explanation for the extraintestinal manifestations seen in NCGS, by hypothesizing aberrant leakage of gluten-related peptides into the systemic circulation. However, the specific gluten peptide triggering mucosal events in NCGS is not clear, with one *in vitro* human study showing gliadin not inducing mucosal inflammation or basophil activation as seen in coeliac disease.[55] Yet, gliadin exposure in gluten-sensitive HLA-DQ8 transgenic mice induced immune activation in the absence of intestinal atrophy, paralleled with increased acetylcholine release from the myenteric plexus resulting in increased muscle contractility and epithelial hypersecretion, with the abnormalities reversed following gluten withdrawal.[56]

Extraintestinal manifestations of NCGS

Neurological manifestations

Even in the absence of coeliac disease, gluten causes neurological manifestations in the form of ataxia, neuropathy and encephalopathy.[57,58] Gluten ataxia is the most common neurological disorder to have been studied (Figure 1).[58] In a case series of 1,000 patients with progressive ataxia, 18% of patients had positive AGA. Among patients with idiopathic sporadic ataxia the prevalence of AGA was 43% compared with 12% in a healthy population and 13% in patients with genetically characterized ataxia.[58] Most patients with gluten ataxia did not have gastrointestinal symptoms, and 60% demonstrated normal histology on duodenal biopsy samples.[58] Furthermore, 39% were negative for deamidated gliadin peptide and anti-TG2 antibodies,[58] but 73% had circulating antibodies to TG6 (a transglutaminase isozyme primarily expressed in the brain).[59] 60% of patients with gluten ataxia had evidence of cerebellar atrophy on MRI and all patients had abnormal magnetic resonance spectroscopy of the cerebellar vermis, suggesting abnormal cerebellar neuronal physiology independent of atrophy (Figure 1a).[58] Postmortem examination of patients with gluten ataxia showed patchy loss of Purkinje cells throughout the cerebellar cortex, but also evidence of inflammation with perivascular lymphocytic cuffing. The clinical response to a GFD depends on the duration of ataxia as prolonged gluten exposure results in irreversible loss of Purkinje cells with atrophy of the cerebellum. Some case reports of patients with established coeliac disease who then developed neurological dysfunction described improvement of the neurological problems with adherence to GFD whilst others did not. None of these reports documented the strictness of adherence to GFD by demonstrating serological elimination of coeliac-disease-specific antibodies, something that is proving to be essential if patients with neurological manifestations are to improve. Importantly, the only study that included patients with NCGS demonstrated that a GFD improves the ataxia (Figure 1b).[60]

Similar observations have been made in gluten-induced peripheral neuropathy.[58] In one study, 34% of patients with idiopathic sporadic neuropathy had circulating AGAs, of which 74% did not have any evidence of enteropathy.[61] In those on a GFD, circulating AGAs were eliminated and neuropathy substantially improved compared with those who maintained gluten consumption;[62] improvement was irrespective of the presence or not of enteropathy. Gluten encephalopathy refers to a combination of intractable headaches often with abnormal brain white matter on MRI. The headache improves after the introduction of a GFD. Possible cognitive deficits associated with such MRI findings remain to be explored. In this group of patients, 43% do not have enteropathy, yet a GFD arrests progression of white matter abnormalities on MRI (Figure 2).[58,63] Whether these neurological

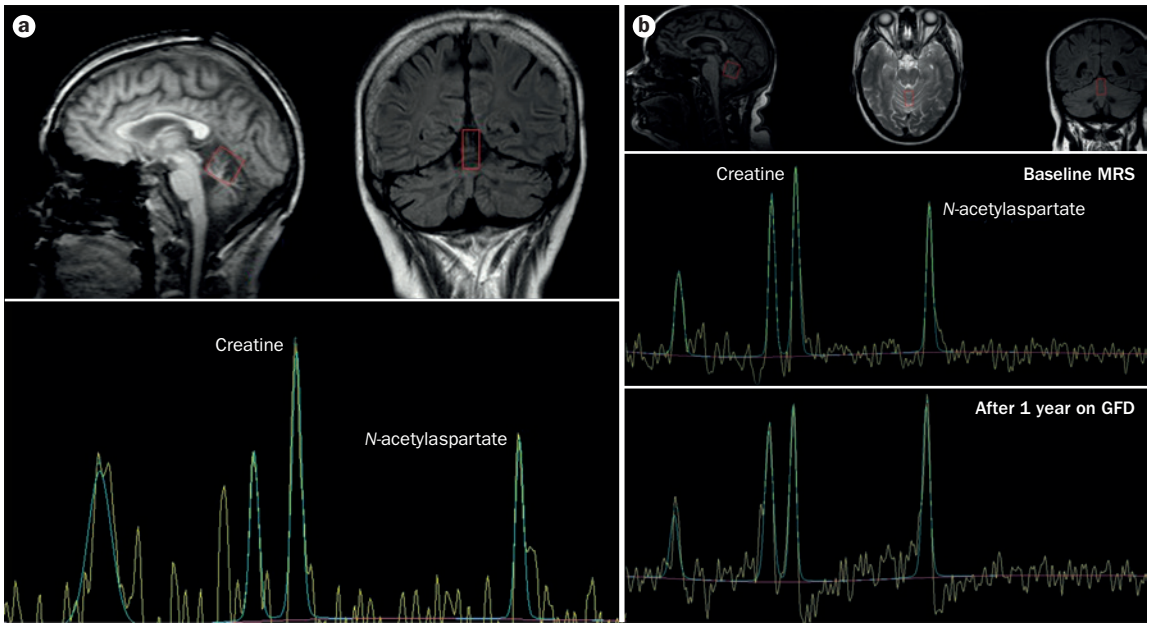

Figure 1 | MRS of the cerebellum in patients with gluten ataxia. These patients had cerebellar ataxia with positive AGA, but no evidence of enteropathy. The voxel was placed in the cerebellar vermis, which is primarily affected in gluten ataxia. In healthy individuals, the ratio of *N*-acetylaspartate:creatine should be >1. **a** | In the first patient, the ratio of *N*-acetylaspartate:creatine was 0.56, which was markedly reduced. **b** | In the second patient the *N*-acetylaspartate:creatine ratio improved from 0.65 to 1.01 after 1 year on a GFD. This increase was associated with clinical improvement of the ataxia. Abbreviations: AGA, antigliadin antibody; GFD, gluten-free diet; MRS, magnetic resonance spectroscopy.

manifestations relate to an autoimmune response primarily directed against TG6 along the same lines as it is directed at TG2 in coeliac disease or TG3 in dermatitis herpetiformis remains to be determined. Importantly, the majority of these patients do not have enteropathy but still improve with a GFD, hence they would currently be considered NCGS.[58]

Psychiatric manifestations

A small DBPC crossover study has suggested that gluten causes depression in patients with NCGS.[64,65] 22 patients with NCGS who randomly received one of three dietary challenges (gluten, whey, or placebo) for 3 days, followed by a minimum 3-day washout before crossing over to the next diet. The mental state at the end of each challenge was assessed using the Spielberger State-Trait Personality Inventory, a validated tool measuring anxiety, depression, anger and curiosity. Short-term gluten exposure specifically induced current feelings of depression with no effect on other indices or on emotional disposition.[64] The authors concluded that the findings could explain why patients with NCGS feel better on a GFD, a suggestion supported by depression not being elevated in NCGS when gluten is eliminated.[66] However, the protein content consisted of 6.6% nongluten proteins (albumin or globulin) alongside the gluten component,[64] raising doubt as to whether the effects can be ascribed to gluten or as a consequence of conflicting substrates, such as amylase trypsin inhibitors (ATIs).

An association between NCGS and schizophrenia has been proposed following reports showing a reduction in psychotic symptoms in a subset of patients on a GFD.[67] Two groups have also shown an increased prevalence of AGA in patients with schizophrenia.[68,69] Individuals with either recent-onset psychosis or multiepisode schizophrenia had increased levels of AGA compared with healthy controls, although deamidated gliadin peptide or anti-TG2 antibodies were not elevated in either psychiatric group, thereby arguing against any association with coeliac disease.[68] In another study, 23.1% of patients with schizophrenia had elevated AGA compared with 3.1% of healthy controls. Anti-TG2 antibodies were present in 5.4% of patients with schizophrenia versus 0.8% of healthy controls, although levels of anti-endomysial antibodies were not increased.[69] Further work has also revealed an increased prevalence of anti-TG6 antibodies in the sera of patients with schizophrenia, which was 21.3% in those who had associated AGA positivity, and 13% in those who were AGA negative; by contrast, the prevalence of anti-TG6 antibodies in healthy individuals was 6%.[70] Future studies need to elucidate the benefits of a GFD in patients with schizophrenia stratified by the presence of AGA and anti-TG6 antibodies.

Fibromyalgia

A case series has shed light on the potential benefits of a GFD in patients with fibromyalgia.[71] In 20 patients with longstanding and fairly debilitating symptom history of fibromyalgia, a GFD was trialled after conventional therapies failed. Coeliac disease was excluded by negative anti-TG2 antibody tests and absence of villous atrophy, although all patients were noted to have increased duodenal IELs. After commencing a GFD, clinical response led to at least one of the following scenarios: remission

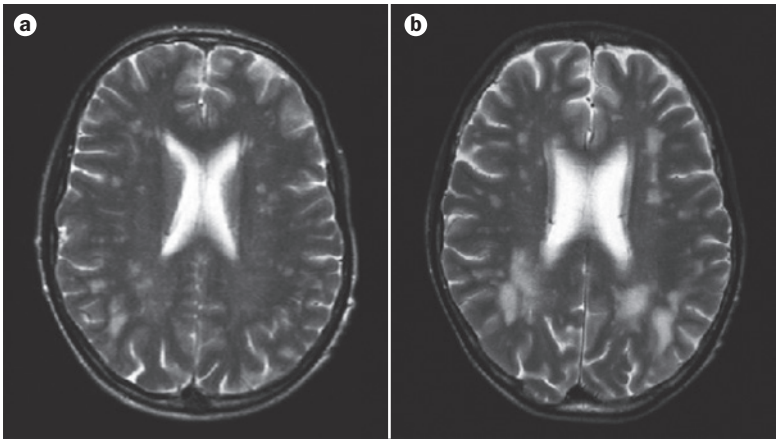

Figure 2 | A head MRI of a 55-year-old patient with intractable headaches and positive AGA (gluten encephalopathy), but no evidence of enteropathy. Initial adherence to a GFD was associated with improvement of the headaches but the patient was unable to adhere to the diet after 3 months. The left scan at baseline shows white matter abnormalities often seen in the context of gluten sensitivity. The right scan 2 years later shows considerable progression of the white matter changes. Strict adherence to a GFD is usually associated with no progression of the white matter changes as well as resolution of the headaches. Abbreviations: AGA, antigliadin antibody; GFD, gluten-free diet.

of fibromyalgia pain criteria; return to work; return to normal life; or discontinuation of opiates. The reintroduction of gluten was followed by fibromyalgia relapse, which subsided upon a GFD.[71] Following on, a case–control study evaluated the effects of a GFD in 97 patients with fibromyalgia and coexisting IBS, in which 58 patients had raised duodenal IELs and 39 had normal duodenal biopsies.[72] Coeliac serology was negative. At baseline, all participants recorded similar poor quality of life and high fibromyalgia and IBS-related symptom scores. After 1 year on a GFD, all outcome measures markedly improved by 26–30% in the increased duodenal IEL group compared with 3–4% in the normal mucosa group. These results stress the potential role of gluten as a trigger of the clinical manifestations of IBS and fibromyalgia and indicate that increased duodenal IEL might be a useful clue to identify those patients who potentially benefit from gluten withdrawal.[72] However, the limitations of these studies are that although coeliac disease was felt to be excluded on the basis of negative serology and absence of villous atrophy, the patients might have had the early stages of coeliac disease (and not NCGS), given that a substantial proportion were HLA-DQ2 and/or HLA-DQ8 positive and showed increased duodenal IEL.

Psoriasis

Although dermatitis and eczema are frequently reported after gluten exposure (Box 1), only the relationship between psoriasis and NCGS has been further evaluated. Psoriasis in patients who are AGA positive can be improved by a GFD.[73] The investigators performed a case–control study involving 33 patients with psoriasis who were AGA positive and six AGA-negative patients. 31 AGA-positive patients were negative for serum anti-endomysial antibodies, with duodenal biopsies showing either increased duodenal IELs or normal biopsy results. After a 3-month period on a GFD, the AGA-positive cohort showed a notable improvement in the psoriasis area and severity index as well as reduction in AGA values. This improvement was not seen in the AGA-negative cohort. When a gluten-containing diet was recommenced there was a deterioration of psoriasis in just over one-half of the AGA-positive patients.[73]

Uncertainties and future directions

Differentiating NCGS from coeliac disease

Despite NCGS seeming to have a reasonably simplistic definition, difficulties do arise in adequately excluding coeliac disease. Firstly, individuals who seek medical attention for gluten sensitivity might already be on a GFD, which in the context of coeliac disease can eliminate serological markers and normalize duodenal biopsies.[74] HLA-DQ typing can be useful in that a negative HLA-DQ2 and HLA-DQ8 result excludes coeliac disease with certainty, which is the case in ~50% of presenting cases.[43] However, if HLA-DQ typing is not readily available or is positive for HLA-DQ2 and/or HLA-DQ8, then patients might need to reintroduce gluten into their diet before formal testing for coeliac disease.[43] Understandably, patients might be apprehensive about undertaking a gluten challenge, which historically entailed 10 g of gluten per day for 6 weeks. However, data have shown that a 2-week challenge of 3 g of gluten per day might suffice and induces histological and serological abnormalities in the majority of adults with known coeliac disease.[75] In this study, the histological abnormalities of villous atrophy were apparent in 68.4% at day 14, whereas serum anti-TG2 antibodies and deamidated gliadin antibody titres rose from 50% at day 14 to 75% at day 28. Using this combined approach, evidence of serological or histological abnormalities for coeliac disease were detected in 89.5%.[75] Nevertheless, this proposed algorithm is yet to be adopted globally.

Alternatively, in those who are unable to perform an oral gluten challenge, an *in vitro* gliadin challenge of duodenal mucosa can help identify cases of coeliac disease, but the availability of this test is limited to selected tertiary-care centres only.[76] Another area of ambiguity is that patients with NCGS can have increased duodenal IELs despite the negative serology. However, these histological changes are not specific and can also occur in coeliac disease.[77] In fact, a proportion of patients with self-reported gluten sensitivity, positive HLA-DQ2 or HLA-DQ8 status, negative coeliac serology, yet increased duodenal IELs will have anti-endomysial and/or anti-TG2 antibodies on the duodenal culture medium, thereby supporting a diagnosis of coeliac disease rather than NCGS.[32,78–81]

To summarize, not only is the best dosage and duration of gluten challenge to investigate patients presenting with self-reported gluten sensitivity uncertain, but international consensus agreement on diagnostically differentiating coeliac disease from NCGS is lacking.[82] This uncertainty can be reflected in the literature in which various groups have undertaken different methodological strategies when investigating self-reported gluten sensitivity and shown the prevalence of coeliac disease to range from

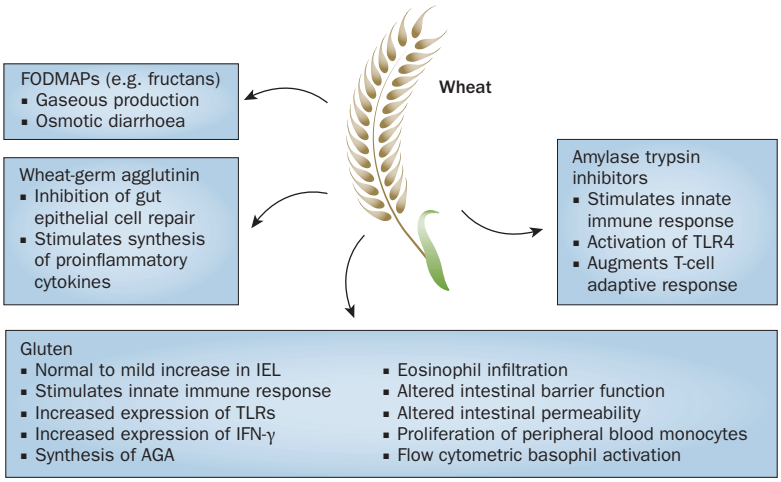

Figure 3 | Proposed effects of wheat-based constituents that trigger clinical symptoms in NCGS. Gluten, FODMAPs, amylase trypsin inhibitors and wheat-germ agglutinin have been identified as causing symptoms in patients with NCGS. Some of the effects attributed to gluten might be caused by nongluten components. Abbreviations: AGA, antigliadin antibody; FODMAP, fermentable oligosaccharide, disaccharide, monosaccharide and polyol; IEL, intraepithelial lymphocyte; NCGS, noncoeliac gluten sensitivity; TLR, Toll-like receptor.

2–45.5%.[16,43,82–85] This variation inevitably leads to some of the studies mentioned earlier open to interpretation as to whether patients with NCGS or coeliac disease are being described. Indeed, at a meeting held in 2014 among experts in the field of gluten-related disorders, the panel regularly raised the issue that future studies and identification of biomarkers in NCGS need to be performed in individuals who are HLA-DQ2 and HLA-DQ8 negative so that potential ambiguity with coeliac disease can be avoided.[86]

Is it gluten or nongluten components?

Gluten is only one of the complex milieu of nutrients present in wheat and other constituents are also capable of triggering symptoms (Figure 3).[87] For example, FODMAPs can cause gastrointestinal symptoms through gaseous production and osmotic diarrhoea,[88–91] and are present in many food products, with fructans commonly present in wheat.[92] In fact, the effects of gluten were questioned after it was demonstrated that individuals with self-reported NCGS already on a GFD further benefited when placed on a low FODMAP diet.[37] Furthermore, the 37 participants in this study then underwent a DBPC crossover trial whereby they received high-dose gluten (16 g gluten per day), low-dose gluten (2 g gluten and 14 g whey protein per day) or control (16 g whey protein per day) for 1 week followed by a washout period of at least 2 weeks before switching to the next diet. The investigators found no specific or dose-dependent effect of gluten.[37] However, recruitment for this study was through media advertisement and many of the individuals presenting with self-reported NCGS were still symptomatic while on their GFD, recording visual analogue scale ratings of up to 60; this finding might not be reflective of those who truly have NCGS. Also, the DBPC crossover trial showed a nocebo response among the three arms, which suggests an anticipatory effect of the crossover study design.

ATIs are natural pesticides that account for ~2–4% of the protein content in wheat. Both *in vivo* and *in vitro* studies have shown ATIs to induce an innate immune reaction through activation of Toll-like receptor 4 on monocytes, macrophages and dendritic cells, leading to release of proinflammatory cytokines within 2–12 h.[93] Furthermore, biopsies from patients with coeliac disease demonstrate that ATIs augment the gluten-specific T-cell adaptive response.[93] However, mouse models that are deficient in Toll-like receptor 4 or its signalling pathway are protected from immune responses upon oral ingestion of ATIs.[93] Therefore, ATIs have been identified as potential new players fuelling inflammation in both coeliac disease and NCGS.[94] Furthermore, wheat-germ agglutinin is a carbohydrate-binding protein that also functions as a natural pesticide. Preliminary studies demonstrate that they can inhibit repair of gut epithelial cells and also stimulate the synthesis of proinflammatory cytokines leading to gastrointestinal symptoms.[95,96]

Therefore, given the current uncertainties regarding which gluten-based constituent is triggering symptoms, it is of some investigators' opinion that patients (particularly those who are AGA negative) should be informed that owing to the absence of diagnostic biomarkers, their condition can be considered as "self-reported" NCGS or noncoeliac wheat sensitivity, or "patients who avoid wheat and/or gluten".[41,97] When available, DBPC rechallenges should use specifically isolated gluten-based constituents, and ideally present them in capsulated form to help prevent any potential ambiguity. In fact, Di Sabatino *et al.*[98] showed that small amounts of purified wheat gluten can trigger symptoms in self-reported NCGS; in a DBPC crossover trial involving 59 participants, intake of 4.375 g of gluten per day for 1 week via gastro-soluble capsules significantly increased overall clinical symptoms compared with placebo in the form of rice starch ($P = 0.034$). Intestinal symptoms such as abdominal bloating and pain, and extraintestinal symptoms such as foggy mind, depression and aphthous stomatitis, were significantly more severe in individuals who received gluten than in those who received placebo.[98] However, whether these findings specifically implicate gluten is still not clear, as although the investigators reported using purified gluten, no additional data were provided on the extraction, in particular whether ATIs were removed.

Recommending GFD in diarrhoea-predominant IBS

Our group reported positive AGAs in ~17% of suspected IBS cases and 12% of the general population, yet the prevalence of biopsy-proven coeliac disease was 4% and 1%, respectively.[99,100] Moreover, a multicentre study has shown positive AGA in 6.5% of suspected IBS cases and 4.8% of healthy controls, yet the prevalence of biopsy-proven coeliac disease was 0.41% and 0.44%, respectively.[101] This finding suggests that the prevalence of AGA (in the absence of coeliac disease) is similar among patients with IBS and healthy controls. Nevertheless, two studies have shown that a GFD can improve abdominal discomfort and stool frequency in patients with diarrhoea-predominant IBS who are AGA positive.[102,103] However, a limitation was

that a subgroup of these patients also had positive serum and intestinal anti-TG2 antibodies, despite the absence of villous atrophy, which would be consistent with a diagnosis of potential coeliac disease as opposed to true diarrhoea-predominant IBS.[104]

In a well-defined cohort of patients with diarrhoea-predominant IBS, without any evidence of coeliac disease, it was shown that those who were HLA-DQ2 and/or HLA-DQ8 positive had accelerated small bowel transit times compared with patients negative for HLA-DQ2 and HLA-DQ8.[105] Furthermore, gluten exposure altered intestinal barrier function in the HLA-DQ2 and/or HLA-DQ8 positive group by reducing the expression of tight-junction proteins and increasing small bowel permeability, leading to increased stool frequency.[53] Further studies are now needed to clarify and elucidate whether a GFD may be a viable treatment option in a select and carefully defined cohort of patients with diarrhoea-predominant IBS previously unaware of the effects of gluten.

Other pathologies in self-reported NCGS

A retrospective study identified that 30% of patients who self-report NCGS had small intestinal bacterial overgrowth.[41] These findings would be biologically plausible in the context of supporting the FODMAP theory as bacteria in the proximal small intestine can act upon ingested fermentable carbohydrates, thus inducing gastrointestinal symptoms. Hence, prospective studies are now required to elucidate the prevalence of alternate aetiologies in patients who self-report NCGS as opposed to them generally being perceived to belong to the spectrum of dietary-related IBS. To cloud matters further, it has been established that there is substantial use of a GFD in patients with IBD, of whom the majority describe an improvement in their gastrointestinal symptoms and disease course.[106] A cross-sectional internet-based survey involving 1,647 patients with IBD identified that 4.9% had a self-reported diagnosis of NCGS, with 19.1% having previously tried a GFD, and 8.2% currently using a GFD.[106] Our group has generated similar results and also found that patients with Crohn's disease who self-report NCGS are markedly more likely to have severe or stricturing disease than those without self-reported NCGS.[107] Furthermore, we evaluated the converse relationship and identified that in 200 patients presenting with self-reported NCGS the majority had perceived dietary-related IBS (98.5%) but a minority had IBD (1.5%); such patients presented with associated alarm symptoms and/or abnormal blood parameters, prompting colonic investigations. This pathophysiological relationship between IBD and self-reported NCGS is unknown but could be due to the physical properties of gluten-based products as a volume effect, or alternatively, there might be a specific immune response that has yet to be explored. Randomized studies are now required to clarify whether a GFD is a valuable option in selected patients with IBD.[107]

Novel diagnostic techniques

Potential biomarkers to help diagnose NCGS have been evaluated. *In vitro* flow cytometry basophil activation tests, based on cell surface expression of CD63, initially showed promising results when compared with DBPC dietary rechallenges, with 86% sensitivity, 88% specificity and 87% accuracy.[108] However, these findings have not been replicated with the use of newer commercial assays, which involve whole blood as opposed to separated leukocytes, nor by other groups.[55,109] Faecal assays, detecting eosinophil cationic protein, have also been proposed showing 65% sensitivity and 91% specificity, but require further validation.[110] Another biomarker for NCGS could be determination of chemokine secretion from peripheral blood mononuclear cells (PBMCs), stimulated by different wheat strains.[111] Preliminary data have shown an increased production of CXCL10 from PBMCs after *in vitro* wheat stimulation in both coeliac disease and NCGS compared with healthy controls.[111] Secretion of CXCL10 is fivefold higher in coeliac disease than in NCGS and the degree of this chemokine increase might be helpful for differentiating coeliac disease from NCGS.[111]

Confocal laser endomicroscopy is able to detect food-associated changes in the intestinal mucosa of patients with IBS.[112] 36 IBS patients with suspected food intolerances and 10 patients with Barrett oesophagus (controls) underwent real-time *in vivo* mucosal imaging using confocal laser endomicroscopy. Following exposure to candidate food antigens (wheat, yeast, milk and soy) via duodenal instillation there were immediate breaks, increased intervillous spaces and increased IEL numbers seen in the intestinal mucosa of 61% of patients with IBS; the most frequently offending food was wheat, accounting for 13 cases. These reactions occurred within 5 min and were not seen in controls. Furthermore, subsequent exclusion diets led to symptomatic improvement of more than 50% at 4 weeks and increased to 74% at 12 months.[112] This innovative technique might prove to be a major breakthrough in diagnosing NCGS, particularly in those who are HLA-DQ2 and HLA-DQ8 negative, although uncertainty might still arise in the HLA-DQ2 and/or HLA-DQ8 positive cohort as such changes will be expected to also occur in coeliac disease. In such difficult cases, detection of intestinal endomysial and TG2 antibodies could prove to be a useful addition.[80,81] However, none of the above-mentioned tests are currently available in routine clinical practice.

Conclusions

Gluten sensitivity and the use of a GFD outside a diagnosis of coeliac disease and IgE-mediated wheat allergy has been termed NCGS. The majority of patients with NCGS describe a constellation of symptoms following gluten exposure, which include intestinal symptoms compatible with IBS, and extraintestinal symptoms such as neurological dysfunction, psychiatric disturbances, fibromyalgia and skin rash. The presence of AGAs supports a diagnosis of NCGS, showing serological disappearance after a GFD, but has limited sensitivity and specificity. Furthermore, confirming NCGS by DBPC dietary rechallenge studies is not routinely available in everyday clinical practice and despite being considered the current gold standard must be viewed with some caution as questions

remain regarding the purity of gluten extract used. As such, most patients presenting with self-reported NCGS should be counselled about the uncertainties surrounding their diagnosis, in particular with regards to whether it is gluten or nongluten components of the grain provoking their symptoms. Future studies need to focus on identifying a diagnostic biomarker to help unravel the enigma that is NCGS.

Review criteria

A literature search was conducted using PubMed and the search term "non-coeliac gluten sensitivity", with the final search performed in March 2015. Systematic reviews, case series, case–control studies and randomized controlled clinical trials were analysed for the creation of this Review, with particular focus on manuscripts published in the past 5 years.

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Author contributions

The authors contributed equally to all aspects in the production of this article.