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Systematic review: noncoeliac gluten sensitivity

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SUMMARY

Background
Noncoeliac gluten sensitivity (NCGS) is a controversial emerging disorder. Despite reported symptoms related to the ingestion of gluten, NCGS remains a diagnosis based on the exclusion of coeliac disease, given the absence of reliable biomarkers.

Aim
To evaluate the prevalence, diagnostic exclusion of coeliac disease and the efficacy of a gluten-free diet (GFD) for NCGS patients.

Methods
A PubMed search was performed up to December 2014. According to consensus diagnostic criteria, NCGS was defined as self-reported gluten intolerance, negative coeliac serology and absence of villous atrophy. Studies evaluating the impact of a GFD on patients with irritable bowel syndrome (IBS) were also included.

Results
Prevalence rates of NCGS (0.5–13%) differed widely. Seventeen studies, including 1561 patients (26 children), met the inclusion criteria for NCGS. HLA haplotypes could not be linked to histology [normal or lymphocytic enteritis (LE)] in 1123 NCGS patients. HLADQ2/DQ8 haplotypes were present in 44% of NCGS patients. After advanced diagnostic techniques in 189 NCGS patients combining LE and HLADQ2/DQ8 haplotypes, 39 (20%) were reclassified as coeliac disease. There was a higher than expected family history of coeliac disease and autoimmune disorders in NCGS patients. A GFD resulted in variable results for variable, but significantly improved stool frequency in HLADQ2 positive diarrhoea-predominant IBS patients.

Conclusions
Prevalence rates for NCGS are extremely variable. A subset of NCGS patients might belong in the so-called 'coeliac-lite' disease. The benefit of a GFD for NCGS patients is currently controversial. HLADQ2 positive diarrhoea-type IBS patients might gain symptom improvement from a GFD.

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INTRODUCTION

Noncoeliac gluten sensitivity (NCGS) was originally described in 1976 and 1978[1, 2] and the first series dates back to 1980,[3] but only since 2010 a rapidly increasing number of studies have called our attention to an apparently novel syndrome or entity, which has challenged physicians and researchers. NCGS is characterised by intestinal and extraintestinal symptoms related to the ingestion of gluten-containing food, in subjects that are not affected by either coeliac disease or wheat allergy.[4–6] NCGS has been reported to affect up to 5–10% of western population and gluten-free foods among noncoeliac patients have grown in popularity.[7–9] Sales of gluten-free food in the US have risen three-fold from 2006 to 2010 and another three-fold increase is expected by 2015.[7] A recent report revealed that about a third of US adults (the highest percentage ever) expressed their willingness to exclude gluten from their diets.[10] This perspective is largely related to the belief that eliminating gluten from the diet increases health and helps with weight loss, or even that gluten can be harmful to every human being. Therefore, NCGS is now recognised as a gluten-related disorder which has clinical, social and economical relevance.

Currently there is an absence of any reliable biomarkers, therefore, NCGS remains a diagnosis of exclusion. It is essential to exclude coeliac disease in clinical practice, based on clinical, laboratory and histological findings.[4–6, 11] The current clinical consensus is that the diagnostic criteria for NCGS should include self-reported gluten intolerance, negative coeliac disease serology (including IgA endomysial antibodies, IgA tissue transglutaminase antibodies and IgG deamidated gliadin peptide antibodies) and the absence of villous atrophy on duodenal histology (whilst on a gluten containing diet).[5, 6, 11] As such, it is accepted that NGCS patients might have an increased number of duodenal intraepithelial lymphocytes (IELs) (>25 IEL/100EC), i.e., lymphocytic enteritis (LE), which represents Marsh 1 lesions (Marsh-Oberhuber) or grade A lesions (Corazza) in the histological classification for coeliac disease.[4–6, 11] LE is a nonspecific histological lesion which may be associated not only with coeliac disease but also to *Helicobacter pylori* infection, small intestine bacterial overgrowth or the use of anti-inflammatory drugs. However, the most frequent cause of LE in patients with positive HLA-DQ2/DQ8 haplotypes after undertaking an exhaustive diagnostic work-up has been coeliac disease, with reported prevalences ranging from 16% to 43%.[12–16] Furthermore, seronegativity is more common in coeliac disease patients without villous atrophy (Marsh 1-2 lesions), but these 'minor' forms of coeliac disease may have similar clinical manifestations to those with villous atrophy[16–18] and may show similar clinical–histological remission with reversal of haematological or biochemical disturbances on a gluten-free diet (GFD).[19, 20]

Among gluten-related disorders, it is critical to make a clear distinction between coeliac disease and NCGS, since both entities could have radically different natural history, prognosis and need for strict long-term gluten avoidance.[21] This recognition and differentiation becomes difficult in patients with negative coeliac disease serology and histological findings (Marsh 1 lesions or LE) not diagnostic for coeliac disease. In this regard, consensus guidelines from the European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) state that a high count of γδ cells (or γδ/CD3 ratio) in immunohistochemical assessment of biopsies or the presence of IgA anti-TG2 intestinal deposits might be specific for CD in patients with LE.[22] In fact, coeliac patients with grade I Marsh mucosal changes show an increase in γδ IELs and deposition of subepithelial anti-tTG IgA when compared to individuals with NCGS.[23] For these hard to diagnose patients, Catassi and Fasano recently proposed that either LE associated with IgA subepithelial deposits or a histological response to a GFD in seronegative patients could be taken as features supporting a diagnosis of coeliac disease.[24] Therefore, this review aims to critically review the available evidence on NCGS, firstly focusing on prevalence figures and diagnostic efforts to rule out coeliac disease and secondly on the results of different dietary interventions for NCGS patients.

METHODS

A literature search was conducted using PubMed, until December 2014. Search terms included 'noncoeliac gluten sensitivity' and 'gluten sensitivity'. The articles returned by the search were selected based on English-language journals. Abstracts were individually reviewed to exclude coeliac patients (referred as gluten-sensitive enteropathy) and patients suffering from other gluten-related disorders (gluten ataxia, autism and neurological symptoms), mostly referenced as gluten sensitivity. Editorials, letters to the editor and reviews were also excluded. Relevant articles (defined by case series with at least four patients) were carefully evaluated through manual review and the reference lists of these articles were examined to include further appropriate articles. Duplicate publications were excluded. According to

current consensus diagnostic criteria,[4–6] NCGS was defined as self-reported wheat or cereals intolerance resulting in gastrointestinal symptoms, which remitted upon gluten withdrawal, with documented exclusion of wheat allergy (IgE skin testing) and coeliac disease (seronegativity and absence of villous atrophy). As for studies dealing with dietary management, only randomised placebo-controlled studies evaluating gluten challenge in NCGS were included. Due to overlap between NCGS and irritable bowel syndrome (IBS), articles evaluating the impact of either gluten-containing or GFD on IBS patients were also included. Search terms included 'gluten' and 'irritable bowel syndrome', with a similar strategy to that mentioned previously.

RESULTS

Prevalence

A paucity of studies, conducted in New Zealand, USA, UK and Italy have been published (Table 1).[8, 25–27] Prevalence rates for NCGS are extremely variable (from 0.5% to 13%), reflecting differences in the recruited target population (from general population or noncoeliac individuals on GFD to referred patients).

Exclusion of coeliac disease

The review and selection process are detailed in Figure 1. From a total of 1207 eligible articles in our initial search, we finally included 17 articles on NCGS[3, 26–41] and 4 articles on IBS.[42–45] One specific study was excluded,[46] since participants were recruited from a preceding study in which subjects with self-reported NCGS were challenged with diets containing varying amounts of gluten.[37] Eighteen of the twenty-one (81%) included articles have been published over the last 4 years. A total of 1561 patients (26 children) met the inclusion criteria for NCGS (self-reported gluten intolerance, negative coeliac serology and absence of villous atrophy on duodenal histology). Diagnostic methods to exclude coeliac disease in each study are detailed in Table 2.

Coeliac genetic study (HLA DQ2/DQ8)

Sixty hundred and eighty-nine NCGS patients (44%) had positive HLA-DQ2/DQ8 haplotypes, which were not evaluated in 52 additional patients (3.3%). As such, coeliac disease could be specifically ruled out by negative HLA-DQ2/DQ8 in 820 patients (52.5%).

However, only two studies fully described the genetic testing for coeliac disease-related HLA haplotypes, including DR5 (*DQA1*05/DQB1*03*), DR3 (*DQA1*05/DQB1*02*), DR7 (*DQA1*0201/DQB1*02*), DR4 (*DQA1*0301/DQB1*03*)[32] and *HLA-DQ2.5 cis* (*DQA1*0501/DQB1*0201*), *HLA-DQ2.5 trans* (*DQA1*0505/DQB1*0301 + DQA1*0201/DQB1*0202*), *HLA DQ2.2* (*DQA0201/DQB0202*) and *DQ8* (*DQA1*0301/DQB1*0302*).[41] In the remaining studies, the definition of positive/negative HLA-DQ2/DQ8 was not clear; furthermore, it was also unclear if HLA DQ2.2 was considered as positive or negative coeliac genetics.

Lymphocytic enteritis (LE)

Three studies, comprising 333 patients of the total cohort (21%) only included patients with LE if their HLA-DQ2/DQ8 was negative.[30, 32, 37] The rate of LE patients with either positive or negative HLA-DQ2/DQ8 haplotypes was only deducible in two studies,[34, 40] in which 71/90 (78%) had HLA-DQ2/DQ8 and LE. In the remaining eleven studies, involving 1123 patients, HLA could not be linked to histological findings: 209 patients (19%) had Marsh 0 biopsy, 253 patients (22%) had LE and a distinction between Marsh 0 and 1 was not specified/evaluated in 661 patients (66%).

Regarding LE patients, count of γδ cells were performed in three studies, but specific results were not detailed[28, 29, 31] (Table 2). In three studies evaluating further techniques for coeliac disease exclusion, 39/189

Table 1 | Prevalence figures for non-coeliac gluten sensitivity (NCGS)

First author, year of publication	Country	Target population	NCGS prevalence
Tanpowpong, 2012[8]	New Zealand	916 children general population	5% GFD (1% CoD)
Sapone, 2012[5]	USA	5896 referred patients	6%
DiGiacomo, 2013[25]	USA	7762 general population free of CoD	0.55%
Aziz, 2014[26]	UK	1002 general population	13% (GFD 3.7%, 0.8% CoD)
Volta, 2014[27]	Italy	12,255 referred patients	3.2%

CoD, coeliac-disease; GFD, gluten-free diet.

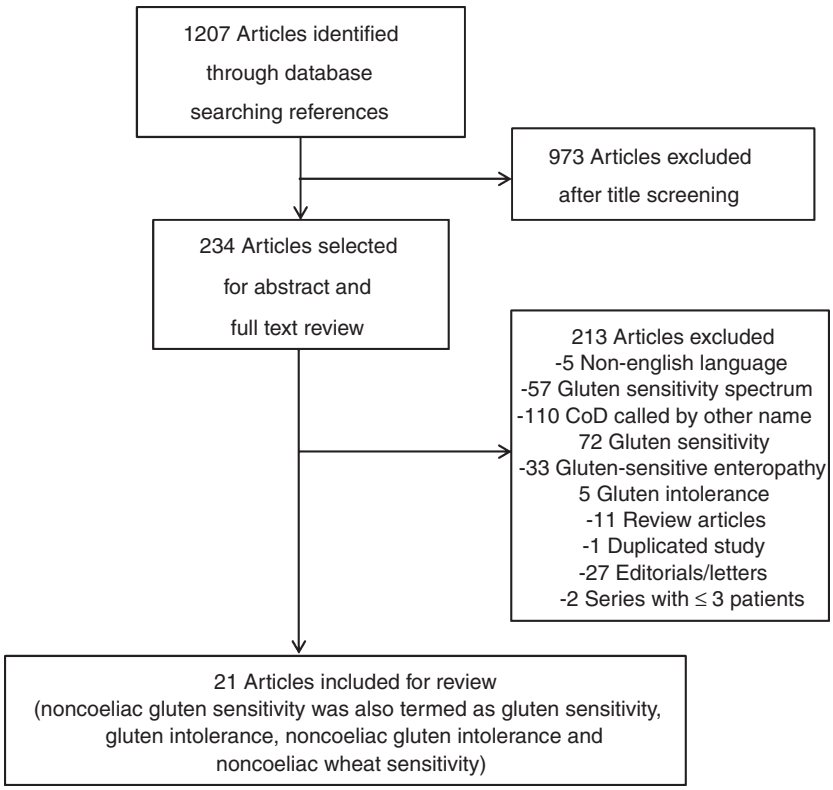

Figure 1 | Flowchart of literature search carried out for this review. CoD, coeliac-disease.

(20%) of NCGS patients showing HLA haplotypes were eventually reclassified as having coeliac disease[26, 34, 36] (Table 2).

Family history of coeliac disease, malabsorption signs and autoimmune disorders

Eight of the 18 studies, comprising 1050 patients, have described the presence of malabsorption signs, such as weight loss, anaemia, low ferritin and vitamin deficiencies (folate, vitamin B12 and vitamin D) in NCGS patients[3, 26, 27, 32, 34, 41, 42] (Table 3). Similarly, family history of coeliac disease and autoimmune disorders were commonly described in some of these studies. These data are detailed in Table 3.

Gluten-free diet for NCGS

So far today, three placebo-controlled dietary interventions in patients with presumptive NCGS have been published. These trials are fully detailed in Table 4. Two of them have been performed by the same Australian group with conflicting results.[30, 37] On a first gluten vs. placebo rechallenge trial, patients who received gluten challenge had more abdominal symptoms than those on placebo[30]; however, in a second trial with a crossover design, there were no differences among high-gluten, low-gluten or placebo challenge.[37] In the run-in period of this latter study, patients received a diet low in fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAPs) while maintaining the GFD. It was noted that, that patients were included in the study if they had described symptomatic improvement on a GFD and had prior exclusion of CD. Significantly, these patients showed a further clinical improvement during the run-in period of the study when they were only on a low FODMAP diet.[37] In these two trials, CD was excluded on the basis of a HLA-DQ2 and DQ8 negative genetic study or a normal duodenal histology (Marsh 0) on patients HLA-DQ2/DQ8 positive. In addition in the second one, IFNγ ELISPOT before and after gluten-challenge was performed, and in only one case was a response similar to that reported in CD.[37]

From another perspective, Carroccio *et al.* reported that NCGS patients could be selected on the basis of a double-blind placebo-controlled gluten challenge.[34] Based on this criterion, 276 of 920 (30%) IBS patients symptom-free on a GFD were considered as NCGS. Two groups of patients were defined, those with wheat sensitivity alone and those suffering from multiple foods

Table 2 | Results of coeliac disease testing in NCGS patients

First author, year of publication	*n*	Positive HLA DQ2/DQ8	Lymphocytic Enteritis (>25 IEL/100 EC)	High count of γδ cells	Other diagnostic techniques*
Cooper, 1980[3]	8	3 (37%)	N.S. 82/mm^2 (normal diet) 54/mm^2 (GFD)	N.S.	None
Kaukinen, 2000[28]	84	41 (51%) 3 patients not available	N.S. (possibly 50% had LE, Figure 1 in the article)	Lower density compared to CoD, but increased compared to controls	None
Sapone, 2010[29]	11	4 (36%)	N.S. (intermediate between CoD and controls)	N.S. (similar to controls)	None
Biesiekierski, 2011[30]	34	19 (56%)	Only included LE patients with negative HLA-DQ2/DQ8	N.S.	None
Sapone, 2011[31]	26	12 (46%) 5 patients not available	22 (84%)	N.S. (lower than CoD)	None
Massari, 2011[32]	262	64 (24%)	29 (11%) 21% HLA-DQ2/DQ8+ were offered a GFD	N.S.	None
Brottveit, 2012[33]	31	31 (100%)	N.S.	N.S.	Negative HLA-DQ2-gliadin tetramer test
Carroccio, 2012[34]	70	53 (75%)	67 (96%)	N.S.	22 (30%) patients had EmA in culture medium of biopsy. The authors admitted they should have been classified as CoD
Volta, 2012[35]	78	36 (46%)	33 (42%)	N.S.	None
Brottveit, 2013[36]	30	30 (100%)	N.S. (possibly 50% had LE, see Figure 3)	N.S.	Negative HLA-DQ2-gliadin tetramer test 3/15 CoD patients initially included as NCGS patients, but further reclassified as CoD patients
Biesiekierski, 2013[37]	37	21 (57%)	Only included LE patients with negative HLA-DQ2/DQ8	N.S.	

Table 2 | (Continued)

First author, year of publication	*n*	Positive HLA DQ2/DQ8	Lymphocytic Enteritis (>25 IEL/100 EC)	High count of γδ cells	Other diagnostic techniques*
Aziz, 2014[26]	200	106 (53%)	NCGS was defined as Marsh 0 to Marsh 1 with negative serology after the gluten-challenge	N.S.	CoD was diagnosed in 7% (n = 14) of the gluten-sensitive cohort after a gluten-challenge with positive coeliac serology and Marsh 1–3 lesions
Caio, 2014[38]	44	N.S.	18 (42%)	N.S.	None
Francavilla, 2014[39]	15	7 (46%)	2 (13%) 4 (26%) not available	N.S.	None
Isasi, 2014[40]	20	18 (90%)	20 (100%)	N.S.	None
Kabbani, 2014[41]	125	31/73 (42%) Not evaluated in 55/128 (43%)	N.S. NCGS was defined by normal biopsy in 92 patients, including Marsh 0 and Marsh 1 lesions	N.S.	None
Volta, 2014[27]	486	160 (33%)	93 (31%)	N.S.	None

CoD, coeliac disease; EC, enterocytes; EmA, anti-endomysial antibodies; GFD, gluten-free diet; IEL, intraepithelial lymphocytes; LE, lymphocytic enteritis, N.S., not stated.

* No study evaluated IgA anti-TG2 deposits for the diagnosis of CoD.

sensitivity including wheat. It is worth mentioning that in the first wheat sensitivity group (n = 70), 14% of patients had a family history of coeliac disease, 75% were HLA-DQ2/8 positive, 96% of patients had LE and finally, 30% had EmA positive in culture medium of biopsy. These data could suggest that 30% of these NCGS patients actually have coeliac disease in the form of a Marsh 1 type lesion.[47]

Effect of a GFD on patients with IBS

Up to now, four studies have addressed the potential role of GFD for IBS, specifically in diarrhoea-predominant IBS.[42–45] The details of these studies are displayed in Table 5. Importantly, IBS patients did not self-report gluten-related symptoms and all had negative coeliac serology. Collectively, a gluten containing diet seems to significantly increase small bowel permeability in HLA DQ2 positive patients,[44, 45] whereas a GFD significantly improves symptoms[43] and reduces stool frequency[42, 45] in the aforementioned subset of patients.

DISCUSSION

This is the first systematic review bringing together evolving evidence on NCGS. Our study demonstrates significant variability in prevalence rates. The main reason for this discrepancy might be NCGS is mostly a self-reported diagnosis, besides using different target populations (general population, noncoeliac individuals on GFD and referred patients) with different inclusion and exclusion criteria. This conflicts with the gold standard for the diagnosis of food allergies and intolerances, which are usually double-blind placebo-controlled rechallenge.[48] Overall, it is complex to ascertain the prevalence or epidemiology of NCGS when the definition of the entity is limited by the absence of a recognised biomarker. To date, four studies with significant differences in design, setting, recruitment and diagnostic criteria[49] have assessed the diagnostic outcomes in self-reported NCGS.[26, 28, 41, 50] As such, the rates of NCGS (52–93%) and coeliac disease (2–42%) as a final diagnosis were extremely variable.[49] Similarly, a recent survey characterising 147 adults with a self-diagnosis of NCGS exhibited coeliac disease was inadequately excluded in 91 patients (61%).[51] Serology was the most performed test (90%), followed by duodenal histology (60%) and HLA haplotypes (28%). No investigation whatsoever (genotype, antibodies and duodenal biopsies) had been carried out in 15% of patients.

Table 3 | Family history of coeliac disease, malabsorption signs, autoimmune disorders and extraintestinal manifestations in patients with NCGS

Author, year publication	*n*	Family history of coeliac disease	Autoimmune disorders	Malabsorption signs/symptoms (%)	Extraintestinal manifestations (%)
Cooper, 1980[3]	8	None	None	Diarrhoea/weight loss (66.6%) Folate deficiency (33.3%)	Oral apthous ulcers (33.3%)
Massari, 2011[32]	77	N.S.	N.S.	Diarrhoea/weight loss (N.S.) Anaemia (19.4%) Iron deficiency (11.6%) Folate deficiency (7.8%)	N.S.
Carroccio, 2012[34]*	70	10 (14%)	N.S.	Anaemia (70%) Weight loss (45%)	N.S.
Volta, 2012[35]	78	N.S.	N.S.	Anaemia (15%)	'Foggy mind' (42%) Tiredness (36%) Eczema/skin rash (33%) Headache (32%) Joint/muscle pain (28%) Limb numbness (17%) Depression (15%)
Aziz, 2014[26]	186	23 (12.4%)	18 (9.7%)	Anaemia (3.3%) Low ferritin (16.2%) Folate deficiency (7.2%) Vitamin B12 deficiency (3.2%) Hypoalbuminaemia (2.8%)	Tiredness (23%) Headache (22%) Joint pains (8%) Confusion (5%) Leg numbness (6%) Rash (6%)
Isasi, 2014[40]†	20	N.S.	9 (45%)	Iron deficiency anaemia (15%)	Tiredness (40%) Migraine (45%) Arthritis (30%) Osteoporosis (10%) Depression (40%) Hypothyroidism (15%) Oral apthous ulcers (10%)
Kabbani, 2014[41]	125	16 (12.8%)	15 (12%)	Diarrhoea/weight loss (24.8%) Iron deficiency anaemia (2.4%) Vitamin D deficiency (16%) Vitamin B12 deficiency (0.8%)	N.S.
Volta, 2014[27]	486	87 (18%)	68 (14%)	Diarrhoea/weight loss (25%) Anaemia (23%) Low ferritin (23%) Vitamin D deficiency (11%) Folate deficiency (11%)	Lack of well being (68%) Tiredness (64%) Headache (54%) Anxiety (39%) 'Foggy mind' (38%) Numbness (32%) Joint/muscle pain (31%) Skin rash (29%) Depression (18%) Dermatitis (18%) Rhinitis (10%)

N.S., not stated.

* Patients with wheat sensitivity alone.

† Patients with fibromyalgia with negative anti-tTG results.

Currently, a diagnosis of NCGS can be considered when symptoms are clearly gluten-related, coeliac disease serology is negative and no villous atrophy is demonstrated.[4–6] However, we have shown in this systematic review that 20% of patients (showing HLA haplotypes, seronegative Marsh 1 lesions and a clinical scenario suggestive of coeliac disease) with a presumptive diagnosis of NCGS may actually belong in the spectrum of coeliac

Table 4 | Results of randomized, placebo-controlled dietary interventions trials in NCGS patients

First author, year of publication	*n* Definition of NCGS	Design	Duodenal histology	Intervention	Response
Biesiekierski, 2010[30]	34 IBS (Rome III) Symptom control on a GFD	DBPC rechallenge trial Gluten ($n = 19$) vs. placebo ($n = 15$)	No histology in 15 DQ2/8- patients; Marsh 0 in DQ2/8+ ($n = 19$ 10 gluten/9 placebo)	GFD *plus* either gluten supplement (Muffins and bread; 16 g/day) or similar placebo, 6 weeks (Gluten analysis showed that it did not contain FODMAPs)	68% gluten vs. 40% placebo worsening of symptoms ($P < 0.001$). Increase in pain, bloating, stool consistency, and tiredness in gluten group ($P < 0.05$). No changes in serum levels of coeliac Abs and intestinal permeability.
Carroccio, 2012[34]	920 IBS (Rome II) Symptom control on a GFD	Crossover design DBPC gluten vs. placebo challenge to diagnose noncoeliac WS	Absence of villous atrophy 90% of patients had LE.	Elimination diet 4 weeks followed by DBPC challenges using capsules containing wheat or xylose.	30% ($n = 276$) of patients considered as WS (reappearance of symptoms on wheat challenge).
Biesiekierski, 2013[37]	40 IBS (Rome III) Symptom control on a GFD	DBPC crossover trial	No histology in DQ2/8- patients Marsh 0 in DQ2/8+ (n = not specified).	(i) 2-weeks run-in period on a diet low in FODMAPs (while on a GFD) (ii) 1 of 3 diet treatments (high-gluten [16 g/day], low-gluten [2 g/day], or placebo) for 1 week (iii) Rechallenge trial: 3-day challenge 1 of three diet treatments for 3 days (washout period of 3 days minimum).	(i) Most patients improved on a low FODMAP diet ($P < 0.001$) (ii) 7-day challenge trial: Reappearance of symptoms: High-gluten, 16%; Low-gluten, 3%; Placebo, 8% (P = N.S.). (iii) 3-day rechallenge trial: No differences between groups. No changes in serum levels of coeliac Abs and other biomarkers.

DBPC, double blind placebo controlled; GFD, gluten-free diet; IBS, irritable bowel syndrome; LE, lymphocytic enteritis; Abs, antibodies; WS, wheat-sensitivity.

disease, which some authors have so-called 'coeliac lite' disease.[52, 53] There are three studies which might be prime example for this controversy. In the first one, conducted in Germany,[42] coeliac disease had been precluded through negative serology and absence of villous atrophy in 102 patients with diarrhoea-type IBS. Of note, 35% of patients were HLADQ2 positive and 30% had coeliac-associated antibodies in duodenal aspirate, whom significantly improved on a GFD and were likely coeliac patients. The second study, from Italy, showed 70 adult NCGS (seronegative without villous atrophy) patients who were identified through a double-blind randomized placebo-controlled wheat trial.[34] However, 94% NCGS patients had LE, 75% HLA DQ2/DQ8 haplotypes and 30% positive anti-endomysium antibodies in the supernatant of biopsy culture.[47] The authors further admitted that these latter 30% of NCGS patients could actually suffer from coeliac disease.[54] Finally, a recent case series

Table 5 | Effect of a gluten-free diet evaluated in patients with diarrhoea-predominant irritable bowel syndrome (IBS-D)

First author, year of publication	Patients	Measurements	Results	Effect of a GFD
Wahnschaffe, 2001[42]	102 IBS-D; negative CoD serology	• IgA and CoD-antibodies (anti-gliadin and anti-tTG in duodenal aspirate) Intraepithelial lymphocytes (IEL) • HLA-DQ2	• 35% DQ2+ • 23% IEL >40% • 30% CoD-antibodies in duodenal aspirate	Six months GFD (n = 26) Significant decrease in stool frequency and IgA levels in duodenal aspirate ($P < 0.05$) in DQ2+ patients with CoD-antibodies in duodenal aspirate
Wahnschaffe, 2007[43]	145 IBS-D	• CoD-serology • Stool frequency • GI symptom scores	• 39% DQ2+ • 37% IgG-CoD-antibodies • 11/41 (26%) IEL >40%	6 months GFD (n = 45) Normalisation of stool frequency and GI symptoms in 60% DQ2+ *plus* CoD-IgG-antibodies+ patients (vs. 12% in DQ2- patients; $P < 0.05$)
Vazquez-Roque, 2012[44]	45 IBS-D	• Mucosal permeability • Biopsy samples small bowel and rectosigmoid for pathology and tight junction proteins. • GI & colonic transit time • HLA-DQ2/8	• Small bowel permeability increased • DQ2/8+ patients had a decrease in ZO-1 protein in rectosigmoid (vs. DQ2/8- patients)	–
Vazquez-Roque, 2013[45]	45 IBS-D; negative CoD serology GFD vs. GCD	• DQ2/8 • Stool frequency • Small bowel & colon transit • Mucosal permeability • *Ex vivo* cytokine production • Tight junction proteins • Small bowel histology (n = 28)	• Increase in intestinal permeability in DQ2/8+ patients on a GCD. • Increase in IL-10, TNFalpha, GM-CSF (but not IFN-gamma) in GCD patients. • No patient had villous atrophy.	4 weeks GFD Significant decrease in stool frequency GCD vs. GFD, P 0.04 DQ2/8+ vs. DQ2/8-, P 0.019 No impact on daily stool form, ease of passage and gastrointestinal or colonic transit

CoD, coeliac-disease; GCD, gluten-containing diet; GFD, gluten-free diet; IBS, irritable bowel syndrome; IBS-D, diarrhoea-predominant IBS.

from Italy showed three adult NCGS patients with gluten-dependent symptoms, HLA DQ2/DQ8 and Marsh 1 lesions with negative or borderline coeliac disease serology, which showed complete remission on GFD.[55] However, biopsy organ culture of the second part of the duodenum showed EMA IgA and anti-tTG IgA antibody-positive results and these patients were reclassified as coeliac patients. Several diagnostic tools have been proposed in consensus recommendations (γδ + IELs, IgA anti-TG2 deposits) for a final diagnosis of coeliac disease in these hard-to-diagnose patients.[22, 24] However, these techniques (characterisation of γδ + IELs,[56] immunohistological detection of IgA anti-TG2, duodenal aspirate or biopsy culture[34, 37, 42, 55] and HLA-DQ2-gliadin tetramer test[33, 36, 57]) are complex and not always part of current clinical practice. Alternatively, both clinical and histological remission on a GFD could be taken as the features supporting a diagnosis of coeliac disease.[24]

Another potential reason for misdiagnosing NCGS in coeliac patients may be under-rating histological lesions due to gluten restriction in diet.[51] Consequently, some authors have recently recommended that a gluten challenge is necessary in this specific subset of NCGS patients to detect changes in serology and/or histology which could be proof of coeliac disease.[26, 28, 41, 50] Recent data have shown that small gluten amounts (≥3 g of gluten/day) for a short period (2–4 weeks) are sufficient to induce both histological and serological changes in the majority of coeliac adult patients.[58] It should be noted that a gluten challenge equates to challenging with gluten, nongluten proteins and fructans (and other factors in oat, rye and barley).

Recent ACG clinical guidelines for coeliac disease recommended HLA DQ2/DQ8 genotyping in seronegative patients with equivocal small bowel histological findings (Marsh I–II) or patients on a GFD in whom no testing for coeliac disease was carried out before GFD.[11] Given that negative HLA-DQ2/DQ8 testing can clearly obviate the need for further testing for coeliac disease, including endoscopy,[41] we fully agree with the inclusion criteria proposed by the Australian group for NCGS studies[30, 37]: negative HLA-DQ2/DQ8 patients or patients with positive haplotypes but normal histology (Marsh 0). For this reason, we propose a diagnostic algorithm for distinguishing coeliac disease and NCGS in patients with a self-diagnosis of gluten-dependent symptoms (Figure 2). We believe patients with a positive HLA-DQ2/DQ8 and/or LE should not be considered as having NCGS unless coeliac disease has been adequately excluded. In this respect, the importance of misdiagnosing NCGS in coeliac disease relies not only on the possibility of a coeliac patient following a nonstrict GFD but also may result in the overestimation of the response to a GFD in NCGS patients. A strict GFD is mandatory for coeliac

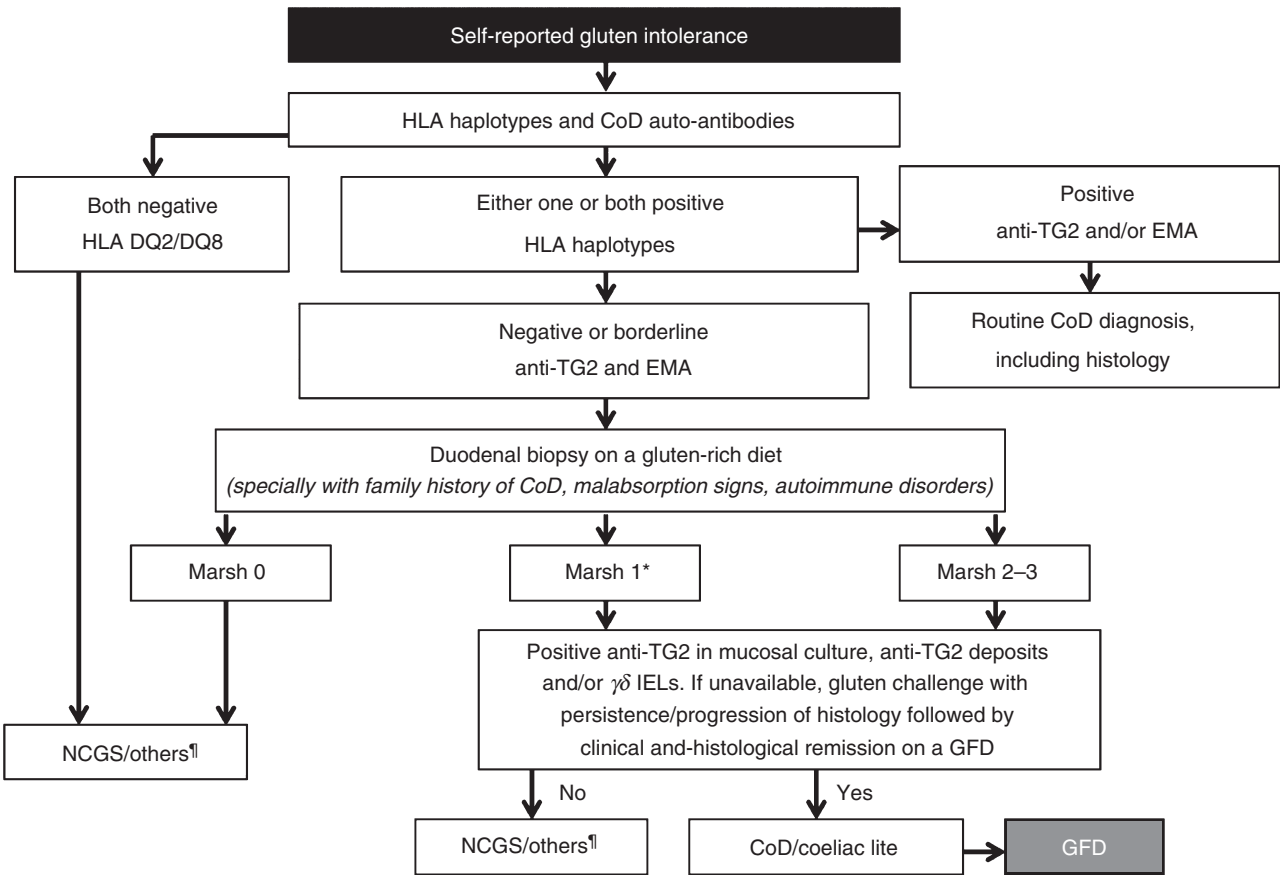

Figure 2 | Diagnostic algorithm for distinction between NCGS and coeliac disease proposed by the authors. *Other causes of lymphocytic enteritis (*H. pylori*, small intestine bacterial overgrowth, NSAIDs,. . .) should have been already ruled out or treated. ¶Potential differential diagnosis: FODMAP-induced symptoms. Anti-TG2: anti-tissue transglutaminase antibodies , CoD: coeliac disease, EMA: anti-endomysial antibodies, FODMAP: fermentable oligo-, di- , mono-saccharides and polyols, GFD: gluten-free diet, IELs: intraepithelial lymphocytes, NCGS: noncoeliac gluten sensitivity.

and wheat allergy patients, although these latter often do not need to restrict rye, barley and oats from their diet. However, it has shown variable efficacy for other gluten-related conditions (NCGS, dermatitis herpetiforme and gluten-sensitive ataxia) or IBS,[59] questioning the need for strict adherence.

Natural history data on NCGS are still lacking. Contrary to coeliac disease, consensus guidelines and review articles have described neither familiar aggregation nor long-term complications, especially malabsorption-related and autoimmune comorbidities, for NCGS.[5, 6, 21] Interestingly, our review shows a remarkable prevalence of familiar history of coeliac disease, malabsorption signs and autoimmune disorders among NCGS patients. As such, the suspicion of misclassification of a subset of coeliac patients as having NCGS is present once again.

As for NCGS, available evidence does not clearly support a GFD for NCGS patients. In the study by Carroccio *et al.*,[34] 30% of NCGS patients had features which could suggest coeliac disease and another subset of patients presented with an allergic profile, within the label of multiple food hypersensitivity. Regarding the studies by the Australian group, they have shown the potential role of FODMAPs as food triggers of gastrointestinal symptoms, but with contrasting results in terms of the culprit agent. In the first study, in which gluten was believed to trigger symptoms, small supplements of gluten or placebo were administered while on a GFD,[30] whereas in the other where FODMAPs were found to induce symptoms, it was a placebo-controlled rechallenge studies, where all food provided was strictly controlled.[37] The Australian studies are also flawed by the high rate of nocebo (anticipatory symptomatic) response, regardless of the nature of the challenge.[37] In addition, no study has addressed the efficacy of low-FODMAP diet beyond a 3-week period, so a placebo response component cannot be ruled out. Furthermore, cereals such as wheat and rye, when consumed in normal quantities, are only minor sources of FODMAPs in the daily diet. Safety concerns about long-term strict adherence to low-FODMAP diet have recently been raised as well, since it might led to insufficient calcium and iron intake[60] and a reduction by 47% of intestinal microbiota.[60, 61] However, nutritional deficiencies may be inherent to any restrictive diet, since coeliac patients have been recently reported to show several nutritional inadequacies[62, 63] or even metabolic syndrome[64] after starting a GFD.

Of note, no study on NCGS has specifically used as the re-challenging agent gluten or gliadin, but bread or a gluten-containing diet. Besides gluten, wheat, barley, rye and their derivatives contain other components that induce symptoms, including carbohydrates (fructans) and other proteins different from gluten, such as amylase-trypsin inhibitors, wheat germ agglutinin and gluten-peptides with opioid effects (exorphins). The potential agents and pathways involved in symptom production after consumption of gluten-containing foods in NCGS are summarised in Figure 3. Thus, there is a concern using the word 'gluten' might not be appropriate and several alternative names have been proposed for this condition (noncoeliac wheat sensitivity[65] or people who avoid wheat and/or gluten[66]).

Unlike coeliac disease, only the innate immune system is seemingly triggered in NCGS.[67] There have been promising reports of altered small bowel intestinal permeability, proliferation of peripheral blood monocytes, the presence of anti-gliadin antibodies, enhanced cytokine induction, and induction of basophil activation in NCGS, although these findings have not yet been confirmed.[67, 68] A recent study has nicely exhibited immediate altered small bowel intestinal permeability (measured through confocal microendoscopy) after exposure to candidate food antigens in IBS patients,[69] including gluten. These findings pose the possibility of NCGS not being a distinctive disease but rather a subgroup of IBS. A recent study published in abstract form, comparing a GFD and a low-FODMAP diet in patients with a presumptive diagnosis of NCGS[70] interestingly showed that NCGS might still be a melting pot with different patients lumped together: 'coeliac lite', NCGS (food hypersensitivity to gluten or nongluten protein mediated through activation of the innate immunity) and patients intolerant to FODMAPs (food intolerance due to carbohydrate malabsorption).[67]

Finally, evolving evidence points towards gluten withdrawal may improve patient symptoms in diarrhoea-predominant IBS.[42–45] These beneficial effects of gluten withdrawal are mostly associated with HLA DQ2 and to a lesser extent, DQ8 genotype. The demonstrated HLA DQ2/DQ8 association suggests an adaptive immune-response to gluten yielding intestinal barrier alterations. Similar to a gluten challenge, a GFD equates to avoiding not only gluten but also nongluten proteins and fructans (and other factors in oat, rye and barley). In this regard, several recent studies have demonstrated the beneficial effect of low-FODMAP diet for every type (diarrhoea-, abdominal pain-, and constipation-predominant) of IBS patient.[71–73] Undoubtedly, we need to learn how to distinguish

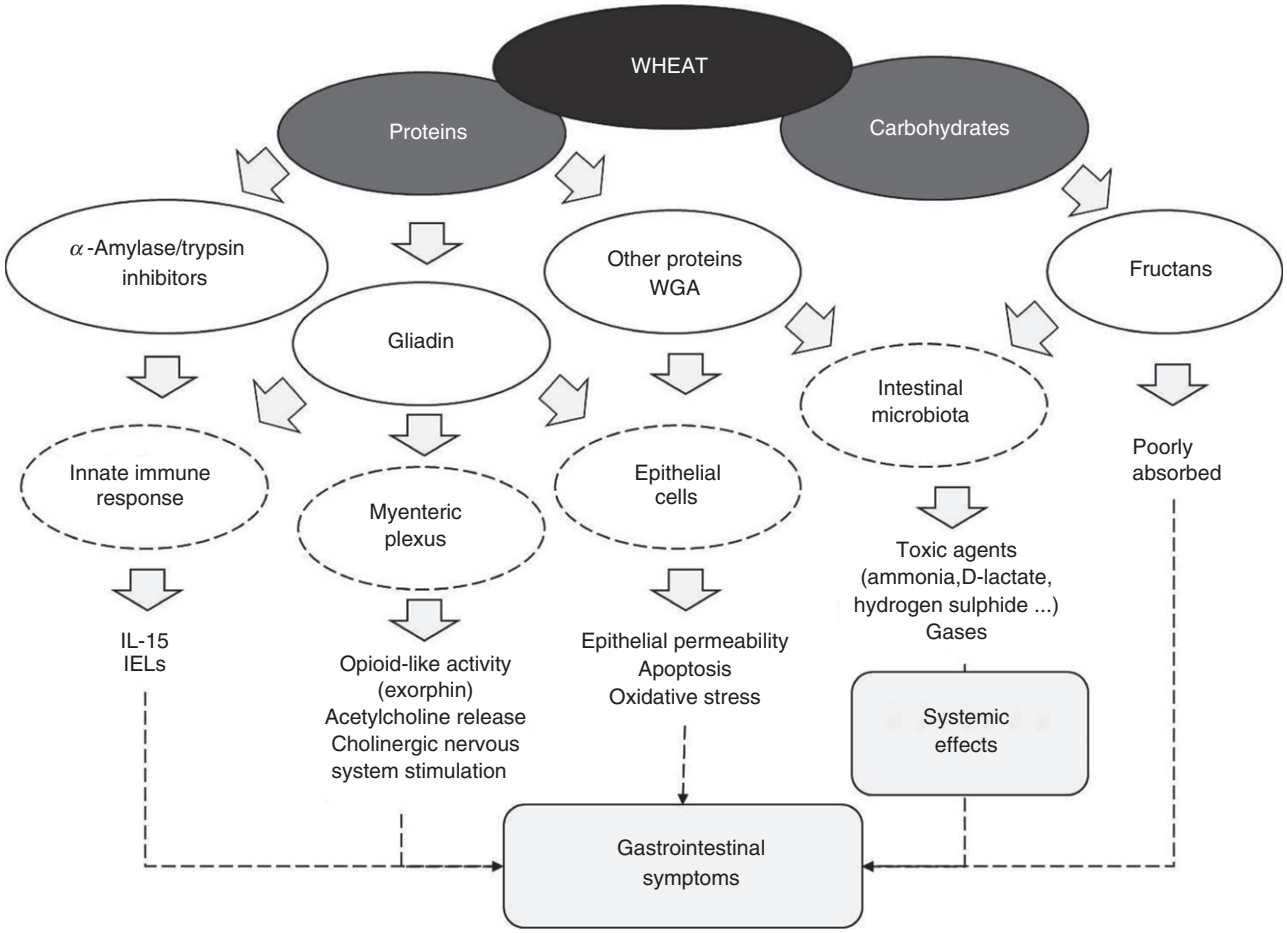

Figure 3 | Potential culprits in wheat and pathogenic pathways for the development of gastrointestinal and extraintestinal symptoms in NCGS. WGA, wheat germ agglutinin; IELs, intraepithelial lymphocytes.

adequately between NCGS and IBS patients, to select the best individualised dietary strategy.

Overall, this review shows variable prevalence rates for NCGS and suggests a subset of patients with NCGS may actually belong in the spectrum of coeliac disease, reinforcing the importance of an adequate exclusion of coeliac disease before a diagnosis of NCGS. Evidence supporting the benefit of either a GFD or a low FODMAP diet for NCGS patients is controversial. Both GFD and low FODMAP diet have recently shown promising result to manage functional gastrointestinal symptoms in IBS and open up an interesting nonpharmacological therapy for these patients. Overlapping features are common between NCGS and IBS patients, so further studies should address how to better diagnose NCGS to select the best choice of dietary therapy.

AUTHORSHIP

Guarantor of the article: Javier Molina-Infante.

Author contributions: Javier Molina-Infante designed the review article. All authors contributed to literature search, data collection, data interpretation, writing, tables, figures and critical revision of the manuscript.

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