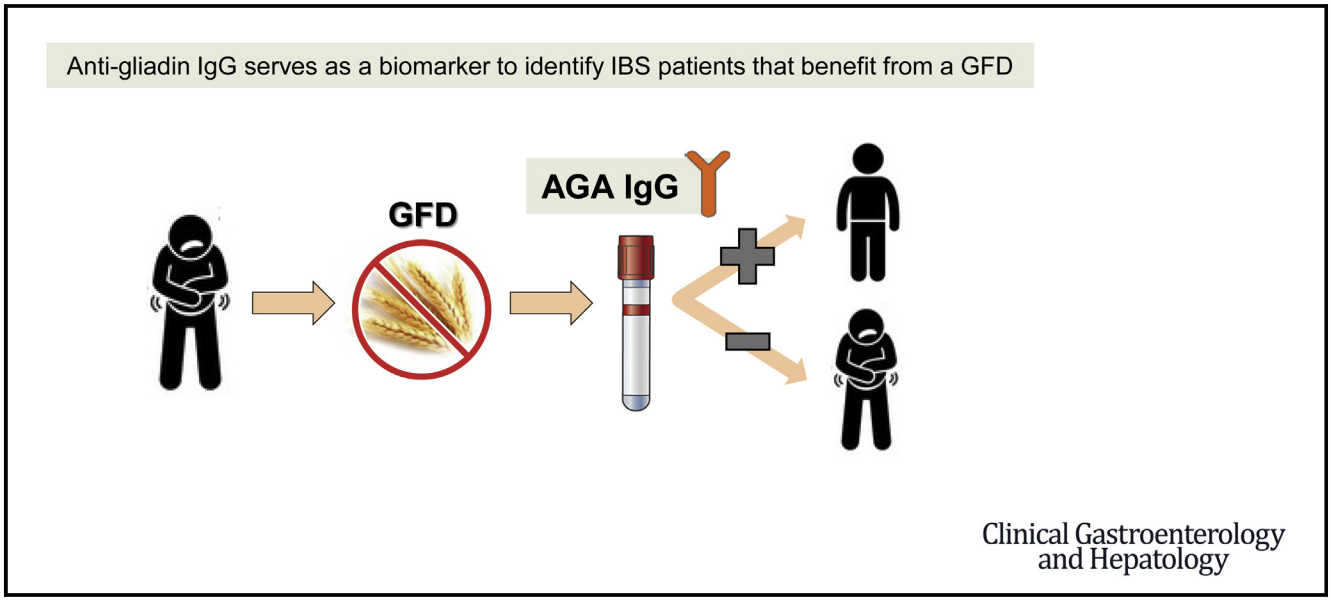
Gluten-Free Diet Reduces Symptoms, Particularly Diarrhea, in Patients With Irritable Bowel Syndrome and Antigliadin IgG

María Inés Pinto-Sanchez,[*] Andrea Nardelli,[*] Rajka Borojevic,[*] Giada De Palma,[*] Natalia Causada Calo,[*] Justin McCarville,[*] Alberto Caminero,[*] Daniel Basra,[*] Alexa Mordhorst,[*] Ekatherina Ignatova,[*] Suzanne Hansen,[*] Melanie Uhde,[‡] Gary L. Norman,[§] Joseph A. Murray,[||] Edgardo Smecuol,[¶] David Armstrong,[*] Julio C. Bai,[¶] Detlef Schuppan,[#] Stephen M. Collins,[*] Armin Alaedini,[‡] Paul Moayyedi,[*] Elena F. Verdu,[*,b] and Premysl Bercik[*,b]

[*]Department of Medicine, Farncombe Institute, McMaster University, Hamilton, Ontario, Canada; [‡]Department of Medicine, Columbia University, New York, New York; [¶]Hospital de Gastroenterologia B. Udaondo, Buenos Aires, Argentina; [§]Inova Diagnostics, San Diego, California; [||]Mayo Clinic, Rochester, Minnesota; [#]Institute of Translational Immunology, Johannes Gutenberg University, Mainz, Germany



BACKGROUND & AIMS: **Many patients with irritable bowel syndrome (IBS) perceive that their symptoms are triggered by wheat-containing foods. We assessed symptoms and gastrointestinal transit before and after a gluten-free diet (GFD) in unselected patients with IBS and investigated biomarkers associated with symptoms.**

METHODS: **We performed a prospective study of 50 patients with IBS (ROME III, all subtypes), with and without serologic reactivity to gluten (antigliadin IgG and IgA), and 25 healthy subjects (controls) at a university hospital in Hamilton, Ontario, Canada, between 2012 and 2016. Gastrointestinal transit, gut symptoms, anxiety, depression, somatization, dietary habits, and**

[b]Authors share co-senior authorship.

Abbreviations used in this paper: AGA, antigliadin antibody; CD, Crohn's disease; ELISA, enzyme-linked immunosorbent assay; FABP2, fatty acid binding protein 2; FODMAP, Fermentable Oligo-, Di-, Mono-saccharides And Polyols; GFD, gluten-free diet; GI, gastrointestinal; GIP, gluten immunogenic peptide; HV, healthy volunteer; IBS, irritable bowel syndrome; LBP, lipopolysaccharide binding protein; OR, odds ratio; tTG, tissue transglutaminase.

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microbiota composition were studied before and after 4 weeks of a GFD. HLA-DQ2/DQ8 status was determined. GFD compliance was assessed by a dietitian and by measuring gluten peptides in stool.

RESULTS: **There was no difference in symptoms among patients at baseline, but after the GFD, patients with antigliadin IgG and IgA reported less diarrhea than patients without these antibodies ($P = .03$). Compared with baseline, IBS symptoms improved in 18 of 24 patients (75%) with antigliadin IgG and IgA and in 8 of 21 patients (38%) without the antibodies. Although constipation, diarrhea, and abdominal pain were reduced in patients with antigliadin IgG and IgA, only pain decreased in patients without these antibodies. Gastrointestinal transit normalized in a higher proportion of patients with antigliadin IgG and IgA. Anxiety, depression, somatization, and well-being increased in both groups. The presence of antigliadin IgG was associated with overall reductions in symptoms (adjusted odds ratio compared with patients without this antibody, 128.9; 95% CI, 1.16–1427.8; $P = .04$). Symptoms were reduced even in patients with antigliadin IgG and IgA who reduced gluten intake but were not strictly compliant with the GFD. In controls, a GFD had no effect on gastrointestinal symptoms or gut function.**

CONCLUSIONS: **Antigliadin IgG can be used as a biomarker to identify patients with IBS who might have reductions in symptoms, particularly diarrhea, on a GFD. Larger studies are needed to validate these findings. ClinicalTrials.gov: NCT03492333.**

Keywords: IBS; biomarkers; antigliadin antibodies; gluten-free diet; FODMAP; gastrointestinal transit; diarrhea.

See editorial on page 2270.

Irritable bowel syndrome (IBS) is characterized by abdominal pain and altered bowel habits in the absence of an organic cause.[1] It is difficult to treat, likely because of heterogeneous underlying mechanisms.[2] Dietary management, based on traditional dietary advice or withdrawal of food triggers, often is offered as first-line treatment. A gluten free-diet (GFD) has gained attention in recent years, although little is known about the exact trigger in wheat-containing foods.[3]

Pathophysiological mechanisms of IBS include neurohormonal dysregulation, immune dysfunction, altered permeability, and impaired bile acid metabolism.[4] Gluten, and nongluten proteins such as α-amylase trypsin inhibitors, can induce innate immune responses, cholinergic activation, dysmotility, and altered gut barrier function.[3,5,6] Recent studies[6,7] in diarrhea-predominant IBS patients have shown increased intestinal permeability that improved after GFD, in agreement with an earlier study suggesting that the diarrhea-predominant IBS patients with immune reactivity to gluten or HLA-DQ2 status are more likely to improve on a GFD.[8]

Identification of a biomarker that predicts clinical response to GFD in IBS patients would improve their clinical management.[4,9] We hypothesized that improvements after a GFD depend on the presence of immune reactivity to proteins of wheat and related cereals, reflected by increased antibodies to the central wheat antigen gliadin (antigliadin antibodies [AGA]). We thus studied unselected, well-characterized IBS patients and assessed their functional and symptomatic responses, as well as putative biomarkers to GFD.

Methods

We performed a single-center study at McMaster University, approved by the Hamilton Integrated Research Ethics Boards. All participants signed the informed consent. The study was registered in ClinicalTrials.gov NCT03492333 after recruitment. All co-authors had access to the study data and reviewed and approved the final version of the manuscript.

Patients

We recruited adult patients with a diagnosis of IBS (ROME III criteria[1,2]), and stratified them according to the presence of antigliadin antibodies (AGA^+ and AGA^-) (Supplementary Figure 1). No patients reported sensitivity to gluten or being on a gluten-restricted diet before the study. Patients with a history of organic diseases, immune deficiency, major abdominal surgery, and those using opioids were excluded. Healthy volunteers (HVs) recruited mainly from students or university employees were enrolled as controls. They had no organic diseases or gastrointestinal symptoms.

Celiac disease (CD) was ruled out in patients and controls by negative tissue transglutaminase (tTG) IgA antibody and deamidated gliadin IgA or IgG antibodies (QUANTA Flash; Inova Diagnostics, San Diego, CA), and by the absence of mucosal atrophy in a duodenal biopsy specimen (Marsh 0 or 1). At least 4 and 2 biopsy specimens were obtained from the second and the first part of the duodenum, respectively.

The use of antidiarrheals, antispasmodics, or laxatives was permitted only if they had been used for at least 1 month before enrollment and the dose did not change during the study. These medications were discontinued 24 hours before motility testing. Probiotics and antibiotics were not allowed for 1 and 3 months before the study, respectively, or during the entire trial. A specialized dietitian (S.H.) provided instructions for a GFD at the first visit (1-hour duration) and assessed GFD adherence at the end of the study. Participants were instructed to continue their dietary patterns otherwise.

We also explored the prevalence of AGA in an ongoing database[10] of new patients referred to the Gastrointestinal Diseases Clinic at McMaster University.

Study Measurements

Gastrointestinal transit (SHAPE study). The colonic shape study[11] measures gastrointestinal (GI) transit using radiopaque markers, clinically used to identify patients with colonic inertia. The patients ingested 1 SITZMARKS capsule (Konsyl Pharmaceuticals, Easton, MD), containing 24 markers, daily for 3 days, and a plain abdominal film was taken on day 4.[11] A cut-off value for increased and decreased transit was established using 25th to 75th and 5th to 95th percentiles of our healthy control values.

Gastrointestinal symptoms. IBS symptoms (diarrhea, constipation, and abdominal pain) were evaluated by the Birmingham IBS symptom questionnaire[12] and stool consistency was evaluated by the Bristol stool scale.[13] Improvement of gastrointestinal symptoms was defined according to a minimally clinically important difference,[9] derived from measures of data variability using one half of a SD. Any patient whose score improved by more than 50% of the Birmingham IBS total score's SD (4.5 of 55; see Results section) was considered to have improved IBS symptoms.

Anxiety, depression, somatization, and well-being. Anxiety and depression were evaluated using the Hospital Anxiety and Depression scale[14] and the State-Trait Anxiety Inventory.[15] Somatization and health-related well-being were assessed by the Patient Health Questionnaire-15 (PHQ-15)[16] and psychological general well-being[17] questionnaires, respectively.

Dietary assessment. The Dietary Questionnaire for Epidemiological Studies (DQESV2, Cancer Council Victoria, Australia)[18] was used to evaluate long-term eating habits; its adapted version was used after a GFD.

Serologic and genetic testing. Total serum IgA was measured to exclude IgA deficiency. Anti-tTG IgA and anti-deamidated gliadin antibodies IgA or IgG (Deamidated Gliadin Antibody IgA–IgG) were tested by immunoassay (QUANTA Flash; Inova Diagnostics). Antigliadin IgA and IgG antibodies were assessed by an enzyme-linked immunosorbent assay (ELISA) (QUANTA Lite, INOVA Diagnostics). A value greater than 20 U tTG, AGA, or DGP antibodies was considered positive. Although tTG and Deamidated Gliadin Antibody tests were performed at recruitment to rule out CD, AGA levels (before and after the GFD) were measured at the end of the study.

For genetic predisposition for CD, we used specific primer polymerase chain reaction (DQ-CD Typing Plus kit; BioDiagene, Palermo, Italy), categorizing patients as having high, moderate, low, or no risk.[19]

Microbiota analysis. Microbiota analysis was performed using Illumina-based 16S-ribosomal RNA gene sequencing as previously described.[20] For details, see the Supplementary Methods section.

Intestinal barrier markers. Plasma lipopolysaccharide binding protein (LBP) (HK315, Hycult Biotech, Plymouth Meeting, PA) and fatty acid binding protein 2 (FABP2) R&D Systems, Minneapolis, MN) were determined by ELISA, following the manufacturers' instructions.

Gluten immunogenic peptides in stool. The concentration of stool gluten immunogenic peptides (GIPs) was measured by ELISA using the iVYDAL GIPs-S kit (Biomedal S.L., Seville, Spain), following the manufacturer's instructions.

Power calculations and statistical analysis. There were no prior data on symptom improvement in IBS patients stratified by AGA levels. A sample size of 25 patients in each arm would have 80% power (5% α level) to detect a 40-percentage-point improvement in the AGA^+ compared with AGA^- subjects, assuming 35% have symptom improvement in the latter group. Statistical analyses were performed using IBM SPSS (SPSS v21.0; SPSS, Inc, Chicago, IL). Our primary outcome was improvement in GI symptoms measured by the Birmingham total IBS score, and secondary outcomes were changes in GI transit, anxiety, depression, somatization, and well-being. Quantitative clinical data were expressed

What You Need to Know

Background

Many patients with irritable bowel syndrome (IBS) perceive that their symptoms are induced by wheat-containing foods.

Findings

In a prospective study of patients with IBS placed on a gluten-free diet (GFD), symptoms, mainly diarrhea, decreased in 75% of those with antigliadin IgG and IgA and in 38% of patients without the antibodies, as assessed by the Birmingham IBS score. The presence of antigliadin IgG, but not strict adherence to a GFD, HLA-DQ2/8 status, or a change in gastrointestinal transit, was associated with an overall reduction in symptoms.

Implications for patient care

Antigliadin IgG can be used as a biomarker to identify patients with IBS who might have reductions in symptoms, particularly diarrhea, on a GFD.

as medians (interquartile range). Analysis of variance, Kruskal–Wallis, with Bonferroni and Dunn–Bonferroni post hoc tests, Mann–Whitney *U*, or chi-squared tests were used as appropriate. Univariate analysis was used to assess the effect of diet on symptoms controlled for baseline. Logistic regression analysis assessed the impact of multiple factors predicting the likelihood of symptomatic improvement with a GFD. We created 2 models, the first included both AGA IgA and IgG, and the second included only AGA IgG, along with HLA DQ2/DQ8 status, GFD adherence, GI transit improvement, and absence of stool GIPs as independent variables. Data were adjusted for age, sex, and body mass index. Comparisons of the microbial profiles were performed using the Kruskal–Wallis test, paired Wilcoxon test, and the Spearman correlation test when appropriate, with Benjamini Hochberg False Discovery Rate correction (5%) for multiple comparisons. A 2-sided test was used and a *P* value less than .05 was considered statistically significant.

Results

Study Population

Seventy-five subjects were enrolled in the study, and 5 of them were excluded for the following reasons: not meeting the Rome criteria (n = 2), enteropathy Marsh III (n = 1), or the presence of GI symptoms in HVs (n = 2) (Supplementary Figure 2). Two patients (1 AGA$^+$ and 1 AGA$^-$) did not comply with the study procedures, dropping out before the intervention. The final study population included 45 IBS patients and 23 HVs (Table 1). Twenty-four IBS patients (53%) and 8 HVs (34.8%) had either AGA IgG and/or IgA at the beginning of the study (Table 1).

Table 1. Demographic Characteristics of IBS Patients and Healthy Volunteers

	IBS AGA$^+$ (n = 24)	IBS AGA$^-$ (n = 21)	HV (n = 23)	*P*
Female	18 (72)	16 (80)	17 (74)	NS
Age, *y*	40 (26–42)	41 (30–57)	30 (21–36)	NS
Caucasian	20 (80)	16 (80)	21 (91)	NS
Smoking	4 (16)	3 (15)	2 (9)	NS
BMI	26 (24–31)	27 (21–29)	23 (21–26)	NS
Alcohol consumption	11 (44)	10 (50)	17 (74)	NS
IBS duration, *y*	5 (2–19)	7 (2–20)	N/A	NS
BMI	27 (24–30)	27 (26–30)	23 (22–27)	NS
IBS subtype				
Diarrhea	10 (45)	8 (35)	N/A	NS
Mixed	2 (9)	3 (13)	N/A	NS
Constipation	8 (37)	6 (26)	N/A	NS
Undetermined	2 (9)	6 (26)	N/A	NS
Family history of CD	5 (20)	5 (25)	3 (13)	NS
HLA DQ2 or DQ8				-
Present	10 (42)	13 (62)	10 (43)	NS
High risk	5 (21)	4 (19)	5 (22)	NS
Moderate risk	4 (17)	8 (38)	4 (17)	NS
Low risk	1 (4)	1 (5)	1 (4)	NS
Absent	12 (50)	8 (38)	12 (52)	NS
Missing data	2 (8)	0 (0)	1 (4)	NS
AGA IgA, *U*	13 (3–192)	7 (0–18)	9 (0–21)	NS
AGA IgG, *U*	25 (21–95)	4 (2–13)	7 (0–27)	NS
tTG, *U*	2 (3)	1 (3)	1 (1)	NS
Increased IELs, >25/field	5	7	NS	NS
Increased IELs + DQ2/DQ8 + family history of CD	2	2	0	NS

NOTE. Data are expressed as n (%) or median (range).
AGA, antigliadin antibody; BMI, body mass index; CD, celiac disease; IEL, Intraepithelial lymphocytes; tTG, tissue transglutaminase.

Effect of a Gluten-Free Diet on Gastrointestinal Symptoms

There were no baseline differences between AGA$^+$ and AGA$^-$ patients in the severity of overall IBS symptoms, constipation, diarrhea, or abdominal pain (Table 2). After a GFD, there were no differences between the 2 groups, however, when adjusted for baseline symptoms, AGA$^+$ patients had less diarrhea than AGA$^-$ patients in a post hoc analysis. By using baseline IBS symptom scores, we calculated that the minimal clinically important difference was a decrease of 4.5 or more of a total of 55 points on the Birmingham score compared with baseline. By using this definition of symptom improvement, we found that after 1 month of a GFD, 18 of 24 (75%) AGA$^+$ patients and 8 of 21 (38%) AGA$^-$ patients improved their overall symptoms (Figure 1*A*). AGA$^+$ patients improved in overall GI symptoms (Z score, 4.2; $P < .001$; ΔAGA$^+$ vs AGA$^-$ $P = .01$) (Figure 1*B*), constipation (Z-2.5; $P = .01$), diarrhea (Z-3.35; $P = .001$), and abdominal pain (Z-3.95; $P < .001$), while AGA$^-$ patients reported improved pain only (Z-2.5; $P = .01$) (Table 2). Similar results were found in the subgroup analysis of patients who strictly complied with the GFD vs those with minor gluten exposure (Supplementary Tables 1 and 2). AGA$^+$ patients were more likely to have an improvement in IBS symptoms than AGA$^-$ patients (odds ratio [OR], 1.96; 95% CI, 1.08–3.55; $P = .017$).

Effect of a Gluten-Free Diet on Gastrointestinal Transit

The median score for GI transit in HVs was 33 hours. We used 2 cut-off values to establish abnormal GI transit, using the 25th to 75th percentile (24–54 h) and the 5th to 95th percentile (14–71 h). By using this approach,

Table 2. Gastrointestinal Symptoms in IBS Patients and Healthy Volunteers

	IBS AGA$^+$			IBS AGA$^-$			HV			IBS AGA$^+$ vs IBS AGA$^-$	
	Before	After	*P*	Before	After	*P*	Before	After	*P*	Before *P*	After *P*
Constipation	6.0 (2.0–7.8)	2.0 (1.0–6.0)	.01	2.0 (0.0–9.5)	1.0 (0.0–2.5)	.19	0.0 (0.0–2.0)	1.0 (0.0–2.0)	.19	.10 .10[a]	.12 .45[a]
Diarrhea	5.0 (2.0–9.8)	2.0 (1.0–4.0)	.001	7.0 (1.5–11.0)	4.0 (1.0–7.5)	.17	0.0 (0.0–2.0)	0.0 (0.0–2.0)	.49	.76 .76[a]	.17 .03[a]
Pain	7.5 (4.0–10.0)	3.0 (2.0–6.0)	<.001	5.5 (3.5–8.0)	3.0 (1.0–5.5)	.01	0.0 (0.0–1.0)	1.0 (0.0–2.0)	.71	.14 .14[a]	.48 .41[a]

NOTE. Data are expressed as median (interquartile range), *P* value before vs after a gluten-free diet.
AGA, antigliadin antibody; HV, healthy volunteer; IBS, irritable bowel syndrome.
[a]Adjusted for baseline symptoms.

abnormal transit was found in 30 of 45 (67%) and 10 of 45 (22%) IBS patients at baseline, respectively. Similar proportions of AGA$^+$ and AGA$^-$ patients had abnormal GI transit at baseline (Table 3).

After the GFD, AGA$^+$ patients were more likely to have normal transit compared with AGA$^-$ patients (OR, 1.75; 95% CI, 1.06–3.06; $P = .04$) when using the 25th to 75th percentile, but the effect was lost when using the 5th to 95th percentile cut-off (Table 3).

Effect of a Gluten-Free Diet on Anxiety, Depression, Somatization, and Well-Being

Anxiety, depression, somatization, and well-being improved after the GFD in IBS patients independently of their AGA status. State and trait anxiety did not change in any subgroup (Table 4).

Fecal Microbiota Composition

Microbial profiles were similar between AGA$^+$ and AGA$^-$ patients, and healthy controls at baseline (Supplementary Figure 3). There were no differences in α-diversity and β-diversity between IBS patients and HVs, before or after the GFD. Microbiota profiles did not correlate with GI symptoms or transit.

Intestinal Barrier Markers

There were no differences in FABP2 and LBP levels between the groups before or after 1 month of a GFD. However, there was a moderate positive correlation between LBP levels and diarrhea in AGA$^+$ patients at baseline (r = 0.47; $P < .01$) (Supplementary Tables 3 and 4), and a moderate correlation between levels of FABP2 and overall GI symptoms (r = -0.43; $P = .04$).

Gluten Immunogenic Peptides in Stool

At baseline, GIPs were found in all subjects, with less than 10% of patients having a very low concentration (<1 ug/g of feces) (Supplementary Table 5). After a GFD, GIPs decreased in all subjects; 81% of AGA$^+$ patients, 67% of AGA$^-$ patients, and 74% of HVs had no measurable GIPs. The remaining subjects had very low or low GIP levels (<10 ug/g of feces), 2 AGA$^-$ patients had moderate GIP levels. Dietitians identified risk of gluten exposure in 32 of 68 subjects, and, of those, GIP was detected in only 13 subjects. There was low agreement between dietitian assessment and GIPs ($\kappa = 0.27$; $P = .015$) (Supplementary Table 6).

Dietary Composition

There was no change in the consumption of food high in Fermentable oligo-, di-, mono- saccharides and polyols (FODMAPs) in AGA$^+$ patients, except for

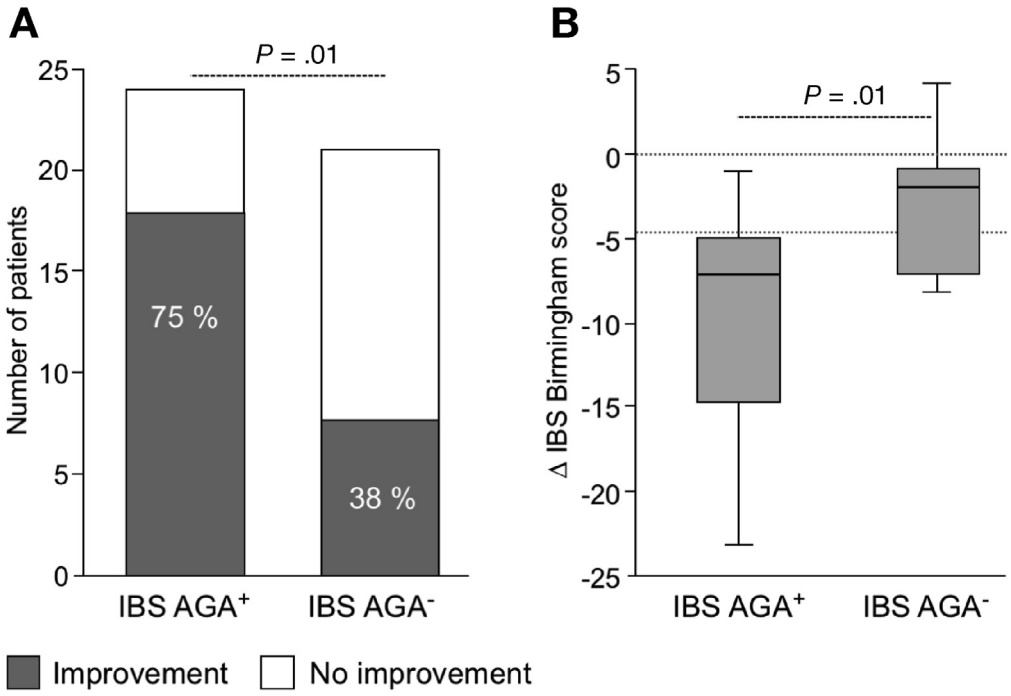

Figure 1. Improvement and change in irritable bowel syndrome (IBS) symptoms. (*A*) Improvement in IBS symptoms (>4.5 points in the total Birmingham score) in antigliadin antibody (AGA)$^+$ and AGA$^-$ patients after GFD. (*B*) Change in IBS symptoms after a gluten-free diet (GFD) compared with baseline in AGA$^+$ and AGA$^-$ patients.

multigrain bread, which also decreased in AGA$^-$ and HV groups. Overall fiber consumption decreased, with no difference between AGA$^+$ and AGA$^-$ patients (Supplementary Results and Supplementary Tables 7 and 8).

Predictors of Symptomatic Response to a Gluten-Free Diet

The presence of increased AGA IgG (>20 U) in IBS patients was associated with a symptomatic response to the GFD (adjusted OR, 128.9; 95% CI, 1.16–1427.8; P = .04). Strict adherence to a GFD, HLA-DQ2/8 status, and changes in GI transit did not predict improvement in symptoms (Table 5).

Validation Cohort: Prevalence of Antigliadin Antibody Positivity in Irritable Bowel Syndrome Patients

Of 4965 patients, 463 (9.3%) were tested for AGA and the test was positive in 104 (22.4%) patients. Of those 463 patients, 328 had a diagnosis of functional GI disorders (Rome III criteria). Of those 328 patients, 71 (21%) had positive AGA. Criteria for IBS were fulfilled by 87 patients, of those, 19 (22%) had positive AGA.

Table 3. Gastrointestinal Transit Before and After 1 Month of a Gluten-Free Diet

	IBS AGA+			IBS AGA-			HV		
	Before, n (%)	After, n (%)	*P*	Before, n (%)	After, n (%)	*P*	Before, n (%)	After, n (%)	*P*
All subjects	24 (100)	24 (100)		21 (100)	21 (100)		23 (100)	23 (100)	
25th–75th percentile of HVs									
Normal transit	9 (37)	14 (58)[a]	.2	6 (29)	6 (29)[a]	1.0	11 (48)	8 (23)	1.0
Accelerated transit	8 (33)	6 (25)	.7	9 (43)	7 (33)	1.0	6 (26)	6 (26)	1.0
Delayed transit	7 (29)	4 (17)	.4	6 (29)	8 (38)	.5	6 (26)	9 (39)	.3
5th–95th percentile of HVs									
Normal transit	18 (75)	22 (92)[b]	.2	17 (81)	18 (86)[b]	1	22 (95)	20 (87)	.6
Accelerated transit	4 (17)	2 (8)	1	4 (19)	3 (14)	1	0 (0)	2 (9)	.4
Delayed transit	2 (8)	0 (0)	.4	0 (0)	0 (0)	1	1 (5)	1 (4)	1

AGA, antigliadin antibody; HV, healthy volunteer; IBS, irritable bowel syndrome.
[a]Odds ratio of normal transit after a gluten-free diet in AGA$^+$ vs AGA$^-$: odds ratio, 1.75; P = .04.
[b]Odds ratio of normal transit after a gluten-free diet in AGA$^+$ vs AGA$^-$: odds ratio, 1.45; P = .6.

Table 4. Effect of a Gluten-Free Diet on Anxiety, Depression, Somatization, and Well-Being

	IBS AGA$^+$ (n = 24)			IBS AGA$^-$ (n = 21)			HVs (n = 23)			IBS AGA$^+$ vs AGA$^-$	
	Before, means (SD)	After, means (SD)	Z P^a	Before, means (SD)	After, means (SD)	Z P^a	Before, means (SD)	After, means (SD)	Z P^a	Before, P^b	After, P^b
HAD Anxiety	8.3 (3.4)	7.1 (3.8)	-2.2 .02	8.7 (4.1)	7.2 (4.3)	-2.2 .02	3.6 (2.6)	3.4 (2.6)	-0.8 .41	.90	.99
HAD Depression	5.8 (3.5)	3.9 (2.9)	-3.0 .002	5.5 (4.1)	4.4 (3.3)	-2.3 .02	1.0 (1.1)	0.9 (1.0)	-0.4 .67	.55	.69
State anxiety	40.9 (11.9)	37.1 (12.2)	-1.8 .06	39.4 (14.7)	37.9 (13.0)	-0.4 .68	25.4 (5.4)	27.0 5.0	-0.0 .98	.52	.84
Trait anxiety	44.6 (11.9)	42.7 (12.4)	-0.8 .38	45.2 (15.1)	43.2 (12.6)	-0.7 .46	28.6 (4.2)	27.0 (5.1)	-1.2 .22	.92	.94
Somatization	13.8 (3.8)	10.2 (4.3)	-2.9 .003	15.2 (4.3)	8.0 (4.5)	-3.5 <.001	4.6 (2.8)	3.6 (2.7)	-2.2 .02	.32	.12
Well-being (total)	61.9 (15.8)	70.4 (18.4)	-2.4 .01	62.0 (22.0)	72.6 (18.1)	-3.0 .002	92.2 (8.5)	89.6 (11.1)	-0.9 .32	.55	.56

AGA, antigliadin antibody; HAD, Hospital Anxiety and Depression scale; HV, healthy volunteer; IBS, irritable bowel syndrome.
[a]Wilcoxon test; Z difference before vs after a gluten-free diet; *P* value.
[b]Mann–Whitney *U* test.

Discussion

Several recent studies have investigated the effects of a GFD in poorly defined non-CD populations, defined as nonceliac gluten (wheat) sensitivity.[21] In our study we took a different approach because we studied a well-defined population of IBS patients to evaluate the effects of a GFD on symptoms and intestinal function. We show that AGA$^+$ patients were more likely to improve symptoms on a GFD compared with AGA$^-$ patients. We did not detect changes over time in the consumption of foods high in FODMAPs, gut microbiota composition, or markers of mucosal inflammation. Overall, our results suggest that in a subgroup of patients with IBS, symptomatic and functional improvement during a GFD may be predicted by the presence of nonspecific AGA.

Notably, 75% of AGA$^+$ patients improved their overall IBS symptoms when maintaining a GFD. This rate is similar to the results of a recent study investigating food-sensitive IBS patients,[22] in which 61% of patients reacted to wheat, followed by yeast, milk, and soy, and symptoms improved after dietary exclusion of the offending food. Because a conventional diagnosis of allergy was absent in these patients, this reaction was termed *atypical food allergy*.

Many studies of IBS have focused on the role of altered motility as a mechanism for symptom generation.[6,8] Our study found that GI transit, which was

Table 5. Predictors of Response to a GFD

	Unadjusted			Adjusted[a]		
	OR	95% CI	*P* value	OR	95% CI	*P* value
Increased AGA (IgA + IgG)	24.5	1.82–328.7	.016	95.4	2.16–4322.1	.03
Increased AGA IgG	35.1	2.46–497.9	.009	128.9	1.16–1427.8	.04
HLA DQ2/DQ8 status	1.52	0.68–2.92	.47	2.39	0.75–7.59	.14
GFD adherence	7.36	0.59–91.4	.12	0.44	0.20–9.70	.60
GI transit improvement	0.84	0.11–6.45	.87	32.7	0.13–789.6	.14
Absence of GIPs in stool	21.9	0.85–565.6	.06	0.77	0.00–15.4	.34
Constant	0.06	–	.05	2.42	0.75–1.40	.84

AGA, antigliadin antibody; GFD, gluten-free diet; GI, gastrointestinal; GIP, gluten peptide; OR, odds ratio.
[a]Adjusted for age, sex, and body mass index.

abnormal in a proportion of IBS patients, was more likely to normalize after GFD in AGA$^+$ patients when using liberal cut-off values of 25th the 75th percentile of values obtained in HVs. However, with more stringent cut-off values (5th–95th percentile), as used in a recent large study of IBS patients,[23] this trend became nonsignificant, although the study was underpowered for this analysis. A previous study in unselected-IBS patients investigating GI transit by scintigraphy showed no overall changes after 4 weeks of GFD.[6] This discrepancy likely is owing to the lack of stratification of patients according to their AGA status or in part to differences in the methodology.

Anxiety, depression, somatization, and lower quality of life, often found in patients with IBS,[10] improved in all patients, independently of AGA status. This is in agreement with a recent study showing that the GFD improved psychiatric comorbidity in IBS patients.[24] Interestingly, somatization improved even in HVs, which is suggestive of a strong placebo effect.

Our data do not confirm previous reports that the genetic predisposition for CD predicts responsiveness to a GFD[8,24] because the HLA-DQ2/DQ8 status was not associated with symptomatic response. In the absence of tTG antibodies, positive AGA indicates nonspecific immune activity toward gluten in many individuals without CD. Half of our IBS patients had increased AGA, mostly the IgG class, with negative IgA anti-tTG, and none of the patients had signs of duodenal mucosal atrophy, which all together effectively rule out CD. However, the AGA$^+$ patients were more likely to improve their symptoms, which is in agreement with an earlier study[8] suggesting that markers of immune reactivity to gluten may be useful as a case-finding strategy in IBS patients, as well as the recently published data on atypical food allergies, in patients with food-sensitive IBS.[22] Although the effect size of GFD was robust, the large CI suggests imprecision, reflecting a relatively small sample size, and thus our finding should be validated in a larger clinical trial.

Gluten intake decreased dramatically in all patients and HVs on a GFD. For rigorous assessment of GFD compliance, we used an experienced dietitian as well as detection of GIPs in stool. The dietitian determined that almost 50% of participants made at least 1 transgression, but only 25% of them had detectable stool GIPs. Although the dietitian evaluated risk for gluten exposure in the long term, the GIP test assessed gluten consumption only during several past days, highlighting the importance of combining these complementary methods. Most importantly, we found that the beneficial effects of the GFD were present even in those patients minimally exposed to gluten, which suggests that strict compliance with a GFD, in contrast to CD patients, may not be necessary in IBS. However, the number of patients who did not adhere strictly to a GFD was small and therefore it is difficult to draw robust conclusions. Larger studies thus are needed to validate these findings.

Dietary patterns differ between IBS subtypes at baseline, suggesting that patients may select the food based on its association with symptoms. We did not detect changes in the overall consumption of foods high in FODMAPs to account for symptomatic improvement, but it should be noted that the food frequency questionnaire did not discriminate levels of fructans, which are found commonly in wheat-containing products and can trigger symptoms in patients with self-reported gluten sensitivity.[25] However, the GFD may not alter fructans levels significantly because gluten-free breads contain similar levels of fructans as regular gluten-containing breads.[26] Similarly, there were no significant shifts in stool microbiota composition or differences in serum FABP2 or LBP levels, which indicates that classic inflammatory processes were not involved. Although there are a paucity of data in IBS, higher FABP2 and LBP levels were found in patients with nonceliac wheat sensitivity,[27] suggestive of barrier damage and systemic immune activation.

The weakness of our study is related to the study design of unblinded treatment, which may lead to an increased risk of bias. However, it generally is accepted that it is difficult to design proper placebo-controlled trials involving diets, especially those with or without the staple food wheat. It thus is possible that there was a placebo effect affecting our results. However, this placebo effect likely had a similar impact on both AGA$^+$ and AGA$^-$ patients. Importantly, both patients and the investigators were blinded to the AGA status because these antibodies were measured after the study was finished. Lastly, we found AGA positivity in half of our 50 unselected IBS patients, which is higher than previously reported in IBS patients[28] as well as in healthy individuals.[29,30] Therefore, we have explored the prevalence of AGA positivity in our large database of newly referred patients, and found that 22% of patients with a strict diagnosis of IBS have positive AGA IgG, which suggests a potential referral bias in the study population.

In summary, our results suggest that a high number of IBS patients benefit from a gluten-/wheat-free diet, and that AGA seropositivity can be used as a biomarker to identify those IBS patients who are more likely to respond symptomatically. The presence of IgG class AGA, but not other factors such as GI transit, genetic predisposition for celiac disease, or compliance with a GFD, predicts the response to wheat exclusion. Importantly, strict compliance with the GFD was not necessary for clinical improvement, suggesting that gluten restriction, rather than strict gluten avoidance, may be sufficient for symptom management in IBS patients. Our results are in agreement with a recent meta-analysis,[31] and original data showing a specific intestinal mucosal response to wheat in a large percentage of IBS patients, followed by improvement on a wheat-free diet.[22] Overall, many

patients with IBS will profit from a GFD, likely owing to several protein antigens/allergens that are present in gluten-containing foods.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Clinical Gastroenterology and Hepatology* at www.cghjournal.org, and at https://doi.org/10.1016/j.cgh.2020.08.040.

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Reprint requests
Address requests for reprints to: Premysl Bercik, Department of Medicine, Farncombe Institute, McMaster University, Hamilton, Ontario, Canada. e-mail: bercikp@mcmaster.ca; fax: (905) 522-3454.

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CRediT Authorship Contributions
MIPS Participated in study design, patient recruitment, data analysis and wrote the manuscript; AN, RB, NCC, KS participated in patient recruitment, data collection; JMC and GDP performed microbiota analysis; AC performed gluten peptides analysis; MU, AA,GLN performed serological markers and critical review of the manuscript; SH performed dietary assessment and GFD compliance; ES, DA, PM, SC, DS, JB participated in the critical review of the manuscript, data interpretation and for important intellectual content; EFV and PB conceptualized, designed and supervise the study, and critically reviewed the manuscript.

Conflicts of interest
This author discloses the following: Gary L. Norman is an employee of Inova Diagnostics, Inc. The remaining authors disclose no conflicts.

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Supplementary Methods

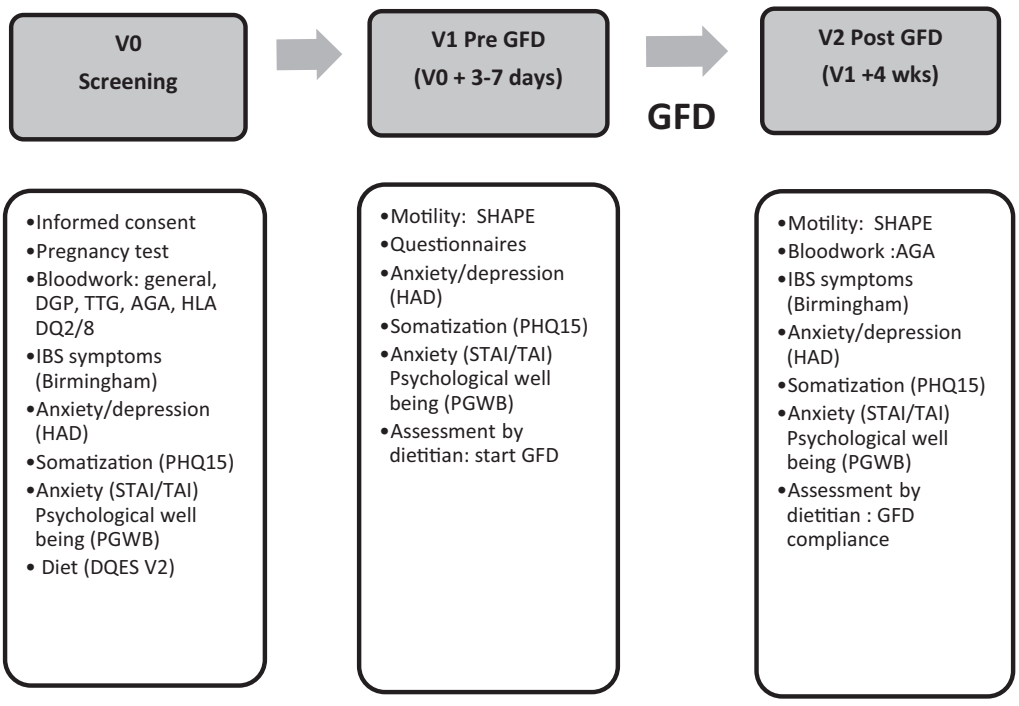
Microbiota Analysis

Custom in-house Perl scripts were used to process the sequences with Cutadapt to trim any over-read, and paired-end sequences were aligned with PANDAseq with a 0.7 quality threshold. If a mismatch in the assembly of a specific set of paired-end sequences was discovered, they were culled. In addition, any sequences with ambiguous base calls also were discarded. Operational taxonomic units were picked using Abundant operational taxonomic units+[3], and sequences were clustered to 97% sequence identity of operational taxonomic units. Taxonomy was assigned at a 0.8 threshold using the Ribosomal Database Project classifier v.2.2 trained against the Greengenes SSU database (2013 release). For all downstream analyses, the obtained operational taxonomic unit table was filtered excluding Root and any sequence that was not present at least 3 times across the entire data set. Calculations of within-community diversity (α-diversity), between-community diversity (β-diversity), significant operational taxonomic units presence, and correlations with symptoms scores and motility scores were run using QIIME and SPSS.

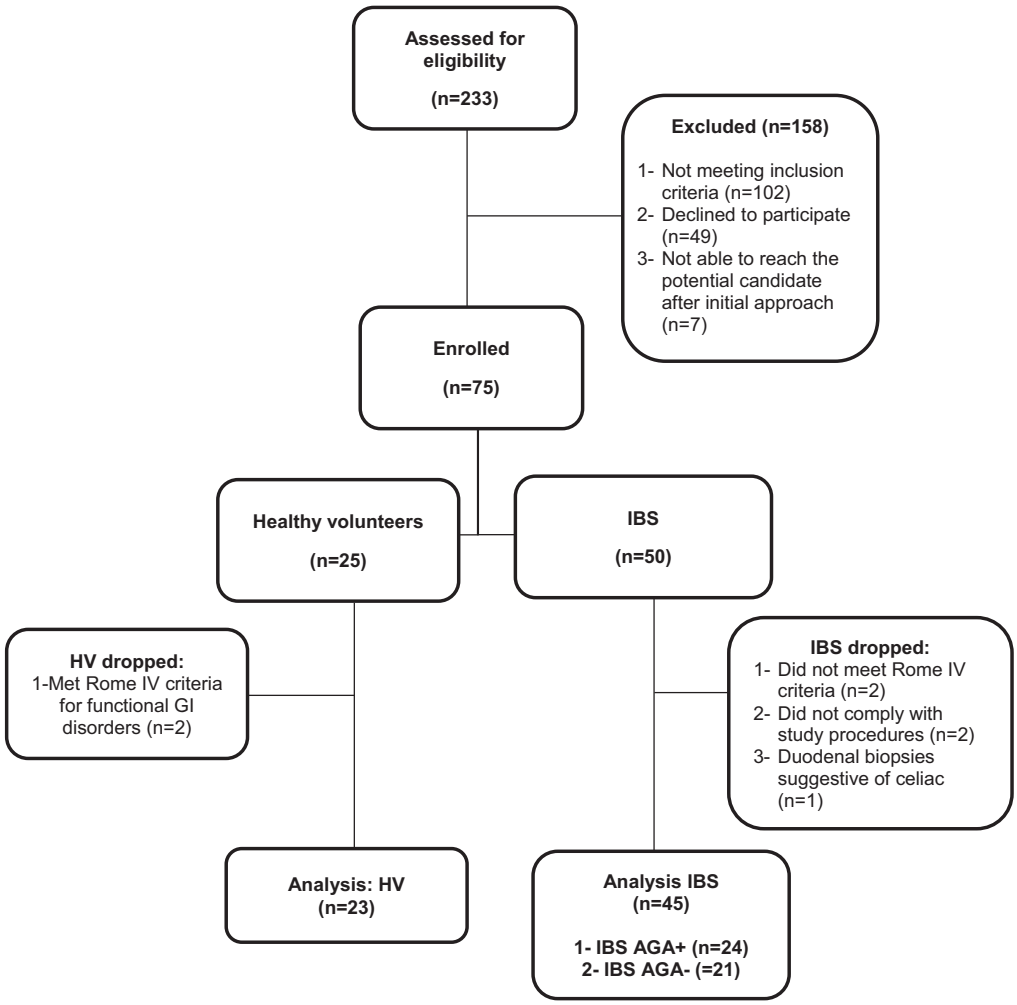
Supplementary Results

Dietary Factors

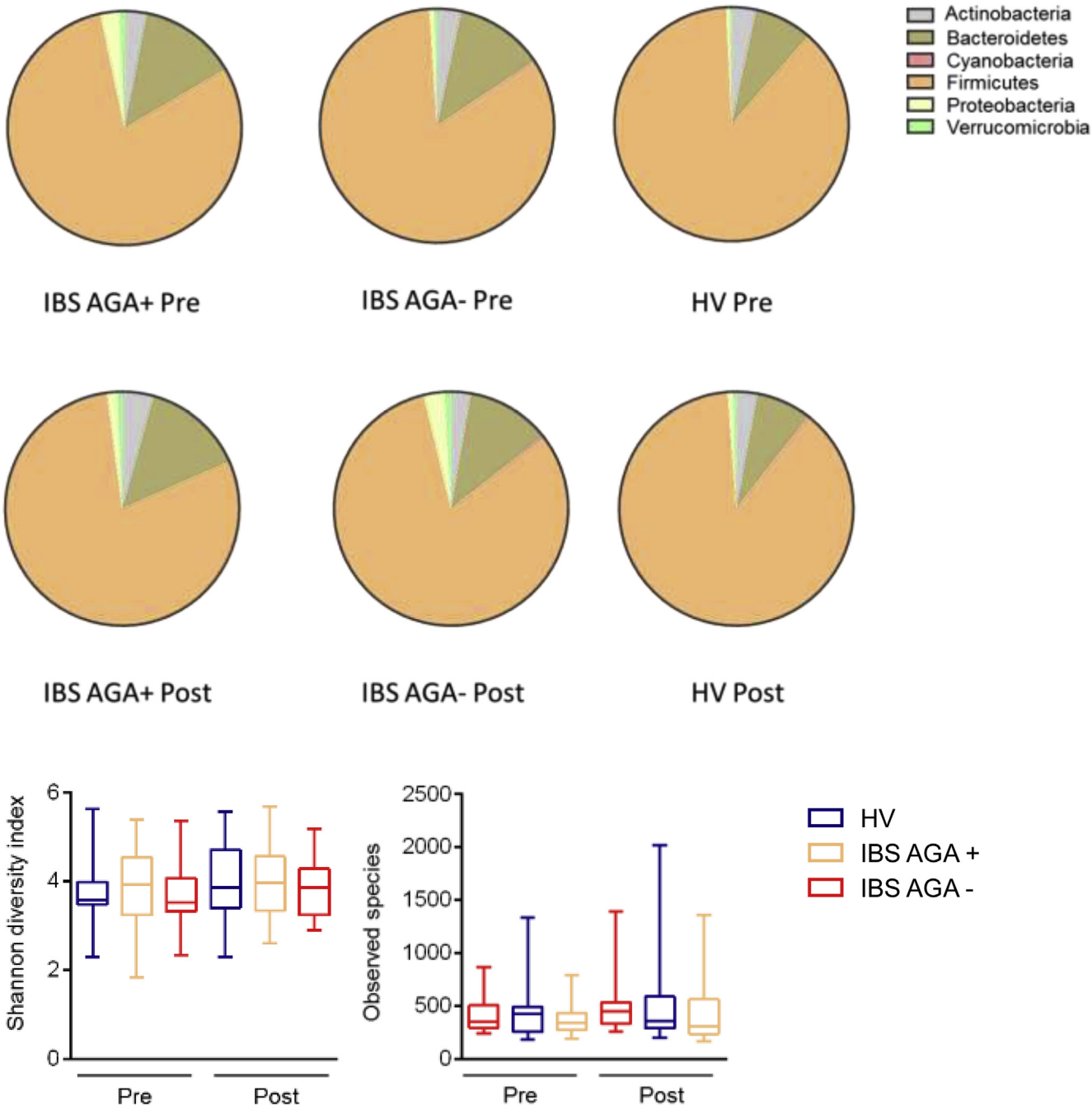
At baseline, both AGA^+ and AGA^- patients had lower fiber intake compared with HVs ($P < .05$), AGA^+ patients also had lower consumption of total protein compared with AGA^- and HVs. After the GFD, AGA^+ patients increased their intake of foods with a high content of vitamin A and C (carrots, lettuce, spinach, and capsicum) and decreased their intake of some cereals (porridge), while AGA^- patients decreased consumption of tinned fruits, multigrain bread, and sausage.



Supplementary Figure 1. Study design. AGA, antigliadin antibody; DGP, deamidated gliadin antibody; DQESV2, dietary questionnaire for epidemiological studies; GFD, gluten-free diet; HAD, Hospital Anxiety and Depression scale; PHQ15, patient health questionnaire-15; STAI, state-trait anxiety inventory; tTG, tissue transglutaminase.



Supplementary Figure 2. Subject recruitment: CONSORT diagram. AGA, antigliadin antibody; GI, gastrointestinal; HV, healthy volunteers; IBS, irritable bowel syndrome.



Supplementary Figure 3. Microbial composition and diversity. AGA, antigliadin antibody; HV, healthy volunteers; IBS, irritable bowel syndrome.

Supplementary Table 1. Improvement of GI Symptoms According to GFD Compliance

	IBS AGA+	IBS AGA-	HV
Overall GI symptoms			
Overall population			
Z	-2.44	-1.33	-1.30
P value[a]	.015	.19	.19
Minimal transgressions			
Z	-3.5	-1.12	-1.06
P value[a]	<.001	.26	.28
Strict compliance			
Z	-3.18	-0.78	-1.06
P value[a]	.001	.43	.28
Constipation			
Overall population			
Z	-2.44	-1.33	-1.30
P value[a]	.015	.19	.19
Minimal transgressions			
Z	-1.61	-0.2	-0.2
P value[a]	.10	.83	.83
Strict compliance			
Z	-0.71	-0.41	-0.21
P value[a]	.47	.66	.83
Diarrhea			
Overall population			
Z	-3.35	-1.44	-0.68
P value[a]	.001	.15	.49
Minimal transgressions			
Z	-2.99	-1.61	-1.34
P value[a]	.003	.10	.18
Strict compliance			
Z	-2.73	-0.59	-1.34
P value[a]	.006	.55	.18
Abdominal pain			
Overall population			
Z	-3.94	-2.5	-0.36
P value[a]	<.001	.01	.71
Minimal transgressions			
Z	-3.43	-1.08	-1.12
P value[a]	.001	.28	.26
Strict compliance			
Z	-3.19	-0.78	-1.06
P value[a]	.001	.43	.28

AGA, antigliadin antibody; GFD, gluten-free diet; GI, gastrointestinal; HV, healthy volunteers; IBS, irritable bowel syndrome.
[a]Wilcoxon test; Z difference before vs after a GFD.

Supplementary Table 2. Intestinal Transit According to GFD Compliance

	IBS AGA$^+$ (n = 24)			IBS AGA$^-$ (n = 21)			HV (n = 23)		
	Before, n (%)	After, n (%)	*P*	Before, n (%)	After, n (%)	*P*	Before, n (%)	After, n (%)	*P*
Strict compliance		17 (71)			13 (62)			14 (61)	
Normal transit	6 (35)	12 (70)	.13	4 (31)	6 (46)	.47	8 (57)	4 (29)	.32
Accelerated transit	4 (24)	2 (12)	.66	6 (46)	3 (23)	.46	3 (21)	5 (35)	.44
Delayed transit	7 (41)	3 (18)	.28	3 (23)	4 (31)	.47	3 (21)	5 (35)	.44
Minimal transgressions		7 (29)			8 (38)			9 (39)	
Normal transit	3 (43)	2 (29)	.99	2 (25)	0 (0)	.49	2 (22)	4 (44)	.39
Accelerated transit	4 (57)	4 (57)	1.0	3 (38)	4 (50)	.68	3 (33)	1 (11)	.61
Delayed transit	0 (0)	1 (14)	.46	3 (38)	4 (50)	.68	4 (44)	4 (44)	1.0

AGA, antigliadin antibody; GFD, gluten-free diet; HV, healthy volunteer; IBS, irritable bowel syndrome.

Supplementary Table 3. LBP and FABP Levels

	AGA$^+$		AGA$^-$		HV			
	Before	After	Before	After	Before	After	P^a Before	P^a After
LBP	11.8 (10.2–17.5)	11.0 (7.9–14.7)	11.7 (8.6–14.3)	11.5 (9.0–12.4)	10.1 (9.0–12.2)	10.1 (8.4–13.8)	.18	.71
FABP2	1.29 (0.6–1.78)	1.20 (0.8–2.2)	1.14 (0.9–1.4)	1.80 (1.4–2.5)	0.85 (0.7–1.3)	1.69 (1.3–1.9)	.38	.62

NOTE. Data are shown as median (interquartile range), expressed in μg/mL.
AGA, antigliadin antibody; HV, healthy volunteer; LBP, lipopolysaccharide binding protein; FABP, fatty acid binding protein.
[a]Kruskal–Wallis test between groups.

Supplementary Table 4. Spearman Correlation

	AGA$^+$	AGA$^-$	HV
LBP/Birmingham total before	r = 0.390; P = .059	r = 0.145; P = .543	r = 0.412; P = .050
LBP/Birmingham diarrhea before	r = 0.476; P = .019	r = 0.152; P = .523	r = 0.352; P = .100
LBP/Birmingham total after	r = 0.192; P = .380	r = 0.325; P = .163	r = 0.171; P = .436
LBP/Birmingham diarrhea after	r = 0.099; P = .653	r = 0.147; P = .535	r = 0.123; P = .577
FABP2/Birmingham total before	r = 0.07; P = .75	r = 0.18; P = .42	r = 0.05; P = .80
FABP2/Birmingham diarrhea before	r = -0.05; P = .81	r = 0.06; P = .77	r = 0.06; P = .78
FABP2/Birmingham total after	r = 0.03; P = .87	r = -0.43; P = .04	r = -0.01; P = .93
FABP2/Birmingham diarrhea after	r = -0.23; P = .31	r = -0.27; P = .22	r = 0.12; P = .56

AGA, antigliadin antibody; HV, healthy volunteer; LBP, lipopolysaccharide binding protein; FABP2, fatty acid binding protein 2.

Supplementary Table 5. GIPs in Stool

	IBS AGA+		IBS AGA-		HV	
GIP, μg/g feces	Before	After	Before	After	Before	After
0.00	0	19	0	14	0	16
0.5	1	3	1	4	1	6
5.0	3	1	5	1	10	1
25.0	13	0	6	2	6	0
75.0	1	0	3	0	1	0
100+	5	0	6	0	5	0

AGA, antigliadin antibody; GIP, gluten immunogenic peptide; HV, healthy volunteer; IBS, irritable bowel syndrome.

Supplementary Table 6. Agreement Between GIPs and the Assessment by the Dietitian

GIP/dietitian[a]	Strict compliance	Poor compliance	Total
No gluten	30	19	49
Gluten peptides, >0.5 ug/g feces	5	13	18
Total	35	32	67

GIP, gluten immunogenic peptide.
[a] κ = 0.27; P = .015.

Supplementary Table 7. Changes in Diet After 1 Month of a GFD

		IBS AGA$^+$		IBS AGA$^-$		HVs	
	Food, *g/d*, median (IQR)	Before GFD	After GFD	Before GFD	After GFD	Before GFD	After GFD
Fruits and vegetables	Tinned fruit	4.90 (0.00–23.08)	3.50 (0.00–21.30)	15.50 (2.10–84.95)	**5.00*** (0.00–23.20)	7.70 (2.80–23.20)	**3.00*** (0.00–12.23)
	Bananas	19.70 (10.1–28.56)	**52.30*** (10.40–81.90)	19.20 (10.17–31.97)	**43.40*** (12.20–63.00)	49.50 (25.20–61.90)	47.71 (36.00–68.50)
	Carrots	1.60 (0.80–8.80)	**9.50*** (1.80–14.50)	4.25 (1.66–6.80)	4.20 (1.70–7.10)	7.60 (5.30–17.10)	**6.90*** (1.20–7.40)
	Lettuce	3.60 (1.20–5.80)	**6.30*** (3.80–9.50)	7.30 (3.38–8.65)	8.78 (5.03–11.20)	6.20 (2.10–10.40)	8.80 (5.10–12.30)
	Spinach	3.10 (0.68–7.10)	**3.90*** (0.20–12.40)	3.51 (1.38–6.88)	3.20 (0.15–4.98)	6.90 (1.30–12.50)	**2.70*** (0.00–5.80)
	Capsicum	0.90 (0.30–2.02)	**2.68*** (0.50–5.20)	2.06 (0.30–3.23)	2.25 (0.60–3.82)	2.02 (1.20–4.50)	2.50 (1.30–3.90)
Grains	Multigrain bread	**0.00** (0.00–60.00)	**0.00*** (0.00–9.47)	30.00 (5.63–60.00)	**3.75*** (0.00–30.00)	15.00 (0.00–60.00)	**0.00*** (0.00–15.00)
	Porridge	7.50 (0.00–48.70)	**0.00*** (0.00–11.93)	0.00 (0.00–19.55)	0.00 (0.00–2.98)	7.50 (0.00–28.40)	0.00 (0.00–16.20)
	Pizza	24.4 (9.10–36.60)	**15.60*** (3.10–19.43)	17.25 (7.53–24.83)	6.20 (0.00–29.30)	23.20 (14.60–63.40)	14.60 (0.00–51.20)
	Cakes	8.05 (1.05–29.43)	**0.00*** (0.00–4.05)	9.70 (2.00–3.90)	**0.45*** (0.00–4.05)	9.50 (4.80–16.80)	**0.00*** (0.00–4.05)
Processed meat	Sausage	3.00 (1.38–11.40)	2.20 (0.00–8.50)	4.30 (1.90–11.05)	**0.75*** (0.00–5.05)	5.90 (1.70–13.70)	**1.80*** (0.00–7.80)
	Meat pie	3.40 (0.00–11.90)	**0.00*** (0.00–3.40)	5.93 (0.00–11.90)	**0.00*** (0.00–3.40)	3.40 (3.40–11.90)	**0.00*** (0.00–3.40)
	Hamburger	10.77 (3.80–13.30)	**0.00*** (0.00–3.80)	13.30 (2.85–13.30)	**0.00*** (0.00–3.80)	13.30 (3.80–26.60)	**0.00*** (0.00–7.62)
Alcohol	Heavy beer	4.20 (0.00–13.50)	**0.00** (0.00–16.70)	**0.00** (0.00–38.90)	**0.00*** (0.00–0.00)	84 (17.20–168.00)	**0.00*** (0.00–27.20)

AGA, antigliadin antibody; GFD, gluten-free diet; HV, healthy volunteer; IBS, irritable bowel syndrome; IQR, interquartile range.

Supplementary Table 8. FODMAP Consumption Before and After a GFD

High FODMAPs	IBS AGA$^+$		IBS AGA$^-$		HV	
Food, *g/d*, median (IQR)	Pre-GFD	Post-GFD	Pre-GFD	Post-GFD	Pre-GFD	Post-GFD
Multigrain bread	0.0 (0.0–60.0)	0.0[a] (0.0–9.5)	30.0 (5.6–60.0)	3.7[a] (0.0–30.0)	15.0 (0.0–60.0)	0.0[a] (0.0–15.0)
Pasta	38.7 (9.4–69.1)	15.50 (2.3–80.6)	33.0 (16.56–49.2)	22.0[a] (1.7–38.7)	51.6 (19.4–83.9)	22.6 (9.7–45.2)
Sweet biscuits	1.5 (0.0–3.9)	0.0 (0.0–3.5)	2.5 (0.4–4.1)	0.0[a] (0.0–1.0)	0.9 (0.0–6.3)	0.0 (0.0–3.4)
Sausages	3.0 (1.4–11.4)	2.2 (0.0–8.5)	4.3 (1.9–11.0)	0.7[a] (0.0–5.0)	5.9 (1.7–13.7)	1.8[a] (0.0–7.8)
Heavy beer	4.2 (0.0–13.5)	0.0[a] (0.0–16.7)	0.0 (0.0–38.9)	0.0[a] (0.0–0.0)	84.0 (17.2–168.0)	0.0[a] (0.0–27.2)
All fiber	15.7 (6.5–24.7)	12.95[a] (8.7–18.4)	18.0 (12.8–21.3)	13.0[a] (10.0–15.3)	20.0 (14.5–27.8)	17.7[a] (12.9–20.3)

AGA, antigliadin antibody; FODMAP, _____; GFD, gluten-free diet; HV, healthy volunteer; IBS, irritable bowel syndrome; IQR, interquartile range.
[a]$P < .05$ compared with before GFD.