Predictors of Clinical Response to Gluten-Free Diet in Patients Diagnosed With Diarrhea-Predominant Irritable Bowel Syndrome

ULRICH WAHNSCHAFFE,* JÖRG–DIETER SCHULZKE,‡ MARTIN ZEITZ,‡ and REINER ULLRICH‡

*Medical Clinic A, Department of Gastroenterology, Endocrinology and Nutritrion, University Hospital Ernst Moritz Arndt Universität Greifswald, Greifswald; and ‡Medical Clinic I (Gastroenterology/Infectious diseases/Rheumatology), Charité-Universitätsmedizin Berlin, Campus Benjamin Franklin, Berlin, Germany

See CME exam on page 769.

See Gass J et al on page 16 for companion article in the July 2007 issue of *Gastroenterology*.

Background & Aims: **Gluten sensitivity might cause abdominal symptoms in the absence of villous atrophy. We examined the prevalence of celiac disease–associated serum antibodies in diarrhea-dominant irritable bowel syndrome (d-IBS) patients and their efficacy in combination with HLA-DQ2 expression to predict the response to gluten-free diet.** ***Methods:*** **HLA-DQA1*0501/DQB1*0201 expression and celiac disease–associated IgA and IgG serum antibodies against gliadin and tissue-transglutaminase were measured in 145 patients with d-IBS, 74 patients with untreated and treated celiac disease, and 57 patients with active IBD. Follow-up antibody levels, stool frequency, and gastrointestinal symptom scores were determined in 41 d-IBS patients (26 women, 15 men; median age, 46 years, range, 30–67 years) who participated in a nonrandomized evaluation of 6 months of gluten-free diet.** ***Results:*** **Increased celiac disease–associated serum IgG, but not IgA, was found in the majority of patients with treated (55%) as in most patients with untreated celiac disease (97%). In d-IBS patients, celiac disease–associated serum IgG antibodies (37%) and HLA-DQ2 expression (39%) were more frequent than in IBD patients (18% and 23%, respectively). After 6 months of gluten-free diet, stool frequency and gastrointestinal symptom score returned to normal values in 60% of d-IBS patients who were positive and in 12% who were negative for HLA-DQ2 and celiac disease–associated serum IgG; both parameters combined yielded positive and negative predictive values of 56% (95% confidence interval, 30%–80%) and 88% (69%–97%), respectively.** ***Conclusions:*** **Celiac disease–associated serum IgG and HLA-DQ2 expression can identify likely responders to gluten-free diet in d-IBS patients.**

Classic celiac disease is a malabsorption syndrome typically diagnosed in patients with diarrhea by the presence of villous atrophy in the small intestine and a rapid clinical or histologic response on a gluten-free diet.[1] Dietary gluten, the storage protein of wheat, induces an abnormal mucosal immune response in susceptible individuals. There is a strong genetic predisposition mostly associated with the markers HLA-DQ2 and HLA-DQ8, which are present in the vast majority of celiac disease patients.[2]

The introduction of serologic tests for celiac disease–associated antibodies against gliadin or tissue-transglutaminase has significantly increased the recognized prevalence of celiac disease, with current estimates ranging from 1 case in 99 to 1 case in 350 people in Western Europe and North America.[3–6] Furthermore, it is now clear that the clinical and histologic spectrum of celiac disease varies widely and might include oligosymptomatic and asymptomatic patients with severe villous atrophy,[7] as well as latent and potential celiac disease patients who are characterized by a normal jejunal mucosa while exposed to a normal gluten-containing diet.[8,9] Latent, in contrast to potential, celiac disease patients at some time point had or develop celiac disease, and both frequently exhibit subtle immunologic abnormalities associated with celiac disease.[10,11] The diagnosis of gluten sensitivity has been proposed to include not only classic celiac disease (gluten-sensitive enteropathy) but also gluten-reactive patients without mucosal lesions.[12] Such patients often experience gastrointestinal symptoms and thus might be clinically indistinguishable from patients with irritable bowel syndrome (IBS), a common condition in which a variable combination of chronic or recurrent gastrointestinal symptoms is present in the absence of organic abnormalities.[13]

We have previously reported the expression of HLA-DQ2 (A1*0501/B1*0201) and the presence of duodenal IgA antibodies against gliadin and tissue-transglutaminase in patients with latent celiac disease.[14] In addition, we found that these markers identify a subgroup of patients with diarrhea-predominant irritable bowel syndrome (d-IBS) who seem to benefit from a gluten-free diet. The determination of antibodies in intestinal fluid is, however, an invasive and non-standard procedure and probably not feasible as routine test. In contrast to serum IgA, serum IgG antibodies against gliadin or tissue-transglutaminase do not decline rapidly after the introduction of a gluten-free diet and often remain increased even after the duodenal mucosa has recovered.[15,16] We therefore extended our earlier study to investigate serum IgG antibodies against gliadin or tissue-transglutaminase in d-IBS patients and examined the

Abbreviations used in this paper: AGA, antigliadin antibody; AU, arbitrary ELISA units; d-IBS, diarrhea-predominant irritable bowel syndrome; DQ2, expression of the HLA-DQ2 alleles A1*0501/B1*0201; ELISA, enzyme-linked immunosorbent assay; TTG, tissue transglutaminase.

1542-3565/07/$32.00
doi:10.1016/j.cgh.2007.03.021

sensitivity and specificity of these markers to predict the clinical response to a gluten-free diet in d-IBS patients.

Methods

Patients

From 1996 to 2004 we examined a total of 583 consecutive patients who attended the gastroenterological outpatient clinic at the University Hospital Benjamin Franklin in Berlin. Patients were classified as having d-IBS if they (1) fulfilled the ROME II diagnostic criteria,[17] (2) had chronic or recurrent diarrhea defined as at least 3 loose bowel movements per day as their main symptom, (3) had no routine laboratory, microbiologic, and endoscopic abnormalities, and (4) had no histopathologic abnormalities exceeding intraepithelial lymphocytosis (Marsh I, this condition precludes celiac disease, which requires at least partial villous atrophy, ie, Marsh IIIA[18]). Patients who had predominantly constipation or who were a first-degree relative of patients with celiac disease were excluded. One hundred forty-five patients (88 women, 57 men; median age, 46 years; range, 17–89 years) fulfilled the inclusion criteria and participated in this study.

We also investigated patients with an established diagnosis of celiac disease according to the revised ESPGAN criteria[18]; patients were classified as having celiac disease if (1) an initial duodenal biopsy had increased intraepithelial lymphocytes, ie, more than 40 intraepithelial lymphocytes per 100 enterocytes, and either partial villous atrophy (Marsh IIIA) or a more severe lesion up to total villous atrophy (Marsh IIIC), and if (2) a clinical and/or histologic remission was documented on a strict gluten-free diet. These patients were subdivided into 2 groups; 30 patients (17 women, 13 men; median age, 50 years; range, 25–65 years) were found to have untreated celiac disease. Forty-four patients (33 women, 11 men; median age, 49 years; range, 18–99 years) had treated celiac disease and underwent follow-up investigation to assess the response to at least 12 months of gluten-free diet.

Data from some patients with IBS or celiac disease have been reported in our earlier study.[14]

As disease control, we studied 57 patients with active IBD (26 women, 31 men; median age, 41 years; range, 29–57 years). Thirty-four were classified as Crohn's disease and 23 as ulcerative colitis. HLA-DQ2 expression was also examined in 62 healthy controls recruited from hospital staff (39 women, 23 men; median age, 38 years; range, 27–62 years).

Informed consent was obtained from all study participants, and the study was approved by the local ethics committee.

Investigations

Each patient underwent an abdominal ultrasound and upper and lower gastrointestinal endoscopy. Distal duodenal biopsies were obtained with biopsy forceps through a conventional forward-viewing endoscope (Olympus, Hamburg, Germany). Three specimens were taken from the duodenal-jejunal flexure for histology and initially stored in 10% formalin. Thereafter, biopsies were embedded in paraffin wax, cut, and stained with haematoxylin-eosin. Histologic features of villus atrophy were assessed in accordance with the revised Marsh criteria,[19,20] and the number of intraepithelial lymphocytes was determined after CD3 immunostaining. Values greater than 40 T lymphocytes per 100 enterocytes were considered to be increased in accordance with the normal range determined in our pathology department. Venous blood samples were drawn from each patient, separated, and stored at −20°C; furthermore, complete blood count, erythrocyte sedimentation rate, thyroid hormones, blood glucose concentration, and chemistry panel for liver, pancreas, and kidney were determined from serum. Stool samples were examined for enteropathogenic bacteria, ova, parasites, and chymotrypsin. Examinations for parasites were also performed in duodenal aspirate.

Gluten-Free Diet in Diarrhea-Dominant Irritable Bowel Syndrome

Forty-one d-IBS patients (26 women, 15 men; median age, 46 years; range, 30–67 years) who were able and willing to follow a gluten-free diet underwent follow-up investigations. Stool frequency was quantified by evaluating the mean stool frequency of the last 4 weeks by means of a questionnaire before and after 6 months of gluten-free diet. In addition, the severity of gastrointestinal symptoms in d-IBS patients was evaluated with a modified symptom score developed and validated by Veldhuyzen van Zanten et al.[21] Our simple questionnaire contained 5 important symptoms typically seen in d-IBS patients: abdominal pain, distention, borborygmus, bloating, and fullness. The severity of each symptom was noted by each patient in reference to a 5-point Likert scale. For each symptom a score value from 1–5 was specified in a questionnaire. Total score values were obtained by summing up the numeric values given for each symptom. As a control, the same symptom score was obtained in 102 healthy volunteers recruited from hospital staff (61 women, 41 men; median age, 36.5 years; range, 27–64 years). Response to gluten-free diet was defined as resolution of diarrhea (ie, less than 3 formed stools per day) and decrease of symptom score values under mean score values + 2 standard deviations of healthy controls.

Celiac Disease–Associated Serum Antibodies

IgA and IgG antibody concentrations against gliadin and tissue-transglutaminase were measured by enzyme-linked immunosorbent assay (ELISA) according to the protocol of Dieterich et al.[22] A pooled serum standard from patients with untreated celiac disease was included in each anti-gliadin and anti–tissue-transglutaminase assay. Results were obtained from a standard curve established according to at least 3 dilutions of this serum standard and converted to concentrations of arbitrary ELISA units (AU). The interassay variation-coefficient was always $<10\%$.

For the evaluation of the anti-gliadin and anti–tissue-transglutaminase IgG and IgA ELISA, serum samples of 29 previously diagnosed patients with untreated celiac disease (18 women, 11 men; median age, 45 years; range, 23–58 years), as well as 113 healthy controls (61 women, 52 men; median age, 48 years; range, 14–94 years) were investigated. None of the control patients had stool abnormalities, received chemotherapy or antibiotics, or was a first-degree relative of patients with celiac disease.

Receiver operating characteristic curve analyses were performed to find the best cutoff levels for each ELISA. For anti-gliadin IgA the cutoff level was established at 36 AU with a specificity of 100% and a sensitivity of 73%. Similar results were found for the anti–tissue-transglutaminase IgA ELISA, which has a specificity of 95% and a sensitivity of 93% (cutoff level, 11

Table 1. Celiac Disease–Associated Serum IgG and IgA, and HLA-DQ2 Expression in Patients With Celiac Disease, d-IBS, and IBD

	Celiac disease		d-IBS		
	Untreated (n = 30)	Treated (n = 44)	Total (n = 145)	DQ2 (n = 57)	IBD (n = 57)
IgG					
AGA	28 (93%)	18 (40%)	29 (20%)	18 (32%)	6 (11%)
TTG	27 (90%)	11 (25%)	33 (23%)	15 (26%)	5 (9%)
AGA/TTG	29 (97%)	24 (55%)	52 (36%)[a]	25 (44%)	10 (18%)
IgA					
AGA	26 (87%)	3 (7%)	3 (2%)	2 (4%)	0 (0%)
TTG	27 (90%)	1 (2%)	4 (3%)	3 (5%)	1 (2%)
AGA/TTG	29 (97%)	3 (7%)	5 (3%)	3 (5%)	1 (2%)
DQ2	30 (100%)	42 (92%)	57 (39%)	NA	13 (23%)

DQ2, expression of the HLA-DQ2 alleles A1*0501/B1*0201; AGA, anti-gliadin antibodies; TTG, anti–tissue-transglutaminase antibodies; NA, not applicable.
[a]$P < .05$ d-IBS vs untreated celiac disease, treated celiac disease, IBD, and 15/62 (24%) healthy controls.

AU). In contrast to the IgA ELISA, sensitivities were higher in the IgG assays (anti-gliadin IgG: cutoff level, 50 AU, 93%; anti–tissue-transglutaminase IgG: cutoff level, 50 AU, 96%) than specificities (anti-gliadin IgG, 86%; anti–tissue-transglutaminase IgG, 93%).

HLA Genotype

Patients were typed for HLA-DQA1*0501 and DQB1*0201 alleles by polymerase chain reaction as described previously.[14]

Statistics

All statistical analyses were performed by using SPSS statistical software, version 11 (SPSS Inc, Chicago, IL). Statistical comparisons were done by the paired and unpaired Student *t* test for continuous data and by Fisher exact test for frequencies. Correlations were tested by the Pearson test. *P* value <.05 was considered significant. Sensitivities, specificities, and predictive values were calculated with 95% confidence intervals.

Results

HLA-DQ2 Expression

All patients with untreated celiac disease and 93% of patients with treated celiac disease were positive for the HLA DQA1*0501/DQB1*0201 gene (Table 1). Thirty-nine percent of d-IBS patients expressed the HLA DQA1*0501/DQB1*0201 gene. Expression of the HLA DQA1*0501 allele without expression of the HLA DQB1*0201 allele was found in 31 of 145 (21%) of the d-IBS patients, and expression of the HLA DQB1*0201 allele without expression of the HLA DQA1*0501 allele was found in 12 of 145 (8%) of the d-IBS patients. Forty-five of 145 (32%) of the d-IBS patients were negative for both alleles. HLA-DQ2 expression was significantly more frequent in d-IBS patients compared with IBD patients (23%, $P = .027$) and controls (24%, $P = .037$; Table 1). Similar proportions of patients with Crohn's disease (8/29, 28%) or ulcerative colitis (6/28, 21%) were HLA-DQ2 positive.

Celiac Disease–Associated Serum Antibodies

IgA antibodies against gliadin and/or tissue-transglutaminase were found in 93% of untreated celiac disease patients but in only 0%–6% of patients with treated celiac disease, d-IBS, or IBD (Table 1). IgA antibody concentrations against gliadin and tissue-transglutaminase correlated weakly ($r = 0.232$; $P < .01$).

IgG antibodies against gliadin and/or tissue-transglutaminase were found in nearly all untreated celiac disease patients and in more than half of the patients with treated celiac disease (Table 1). IgG antibody concentrations against gliadin and/or tissue-transglutaminase were higher in untreated celiac disease patients than in treated celiac disease patients or patients with d-IBS (each $P < .05$, Figure 1). Thirty-seven percent of d-IBS patients had increased IgG antibody levels against gliadin and/or tissue-transglutaminase; this proportion was significantly lower than in patients with untreated or treated celiac disease (each $P < .05$) but higher than in IBD patients (18%, $P < .01$). Similar proportions of patients with Crohn's disease (5/29, 17%) or ulcerative colitis (5/28, 18%) had celiac disease–associated serum IgG antibodies.

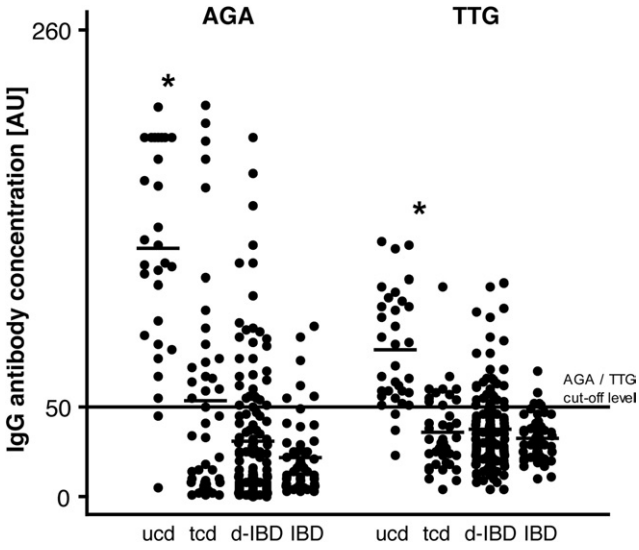

Figure 1. IgG antibody concentrations (AU) against gliadin and tissue-transglutaminase in patients with untreated celiac disease (*ucd*), treated celiac disease (*tcd*), d-IBS, and patients with active IBD. *$P < .05$ ucd vs tcd, d-IBS and IBD.

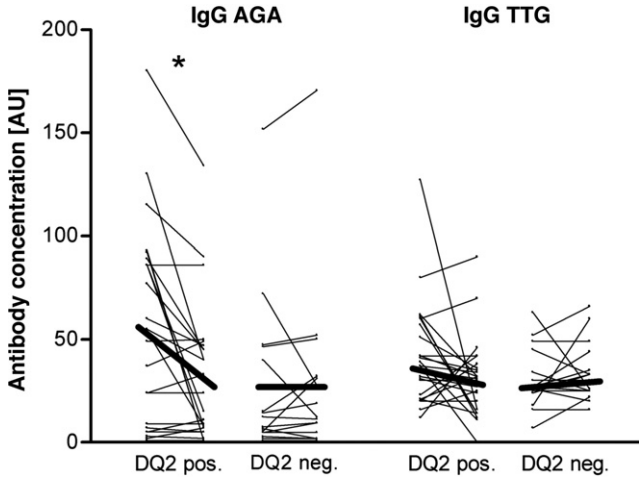

Figure 2. Celiac disease–associated IgG in patients with d-IBS before and after gluten-free diet. Serum IgG antibody concentrations against gliadin (*AGA*) and tissue-transglutaminase (*TTG*) in 25 HLA-DQ2–positive and 16 HLA-DQ2–negative patients with d-IBS before and after 6 months of gluten-free diet. *Bar* denotes mean changes. *$P < .05$.

Increased IgG antibodies against gliadin and/or tissue-transglutaminase were significantly more frequent in d-IBS patients who express HLA-DQ2 than in HLA-DQ2–negative d-IBS patients ($P < .05$). No significant correlation was found between increased IgG antibodies against gliadin and/or tissue-transglutaminase and HLA-DQ2 expression in IBD patients.

Serum IgG antibody concentrations against gliadin and tissue-transglutaminase did not correlate ($r = .086$, $P = .153$). Of 145 d-IBS patients, 29 and 33 patients had increased IgG antibodies against gliadin and against tissue-transglutaminase, respectively, and only 10 patients had increased serum IgG against both antigens (Table 1).

Response to Gluten-Free Diet

IgG antibody concentrations against gliadin decreased from 45.3 ± 7.5 (mean arbitrary units ± standard error of the mean) to 29.9 ± 5.8 ($P < .01$), and IgG antibody concentrations against tissue-transglutaminase decreased from 38.6 ± 7.5 to 32.2 ± 5.8 (NS) in 41 d-IBS patients after gluten-free diet. Decreased concentrations of celiac disease–associated IgG after gluten-free diet were only seen in HLA-DQ2–positive d-IBS patients but not in HLA-DQ2–negative d-IBS patients (Figure 2).

Table 2. HLA-DQ2 Expression and Celiac Disease–Associated IgG in Patients With d-IBS Stratified According to Duodenal Intraepithelial Lymphocytes

	n	DQ2	IgG AGA	IgG TTG	IgG AGA/TTG
<40 IEL/100 enterocytes	30	20 (66%)	9 (30%)[a]	7 (23%)	12 (40%)
≥40 IEL/100 enterocytes	11	5 (45%)	7 (64%)	3 (27%)	8 (72%)

DQ2, expression of the HLA-DQ2 alleles A1*0501/B1*0201; AGA, anti-gliadin antibodies; TTG, anti–tissue-transglutaminase antibodies; IEL, intraepithelial lymphocytes.
[a]$P < .05$.

Table 3. Normalization of Abdominal Symptoms and Stool Frequency in Patients With d-IBS Stratified According to Increased IELs, Celiac Disease–Associated IgG, and HLA-DQ2 Expression

		Normalization of		
	n	Symptom score	Stool frequency	Both
≥40 IEL/100 enterocytes				
Positive	11	5 (45%)	5 (45%)	4 (36%)
Negative	30	15 (50%)	11 (37%)	8 (27%)
DQ2				
Positive	25	17 (68%)[a]	13 (52%)[b]	11 (44%)[b]
Negative	16	3 (19%)	3 (19%)	1 (6%)
IgG (AGA/TTG)				
Positive	20	14 (70%)[b]	10 (50%)	9 (45%)[b]
Negative	21	6 (29%)	6 (29%)	3 (14%)
DQ2 and IgG (AGA/TTG)				
Positive	16	14 (93%)[a]	10 (60%)[b]	9 (60%)[b]
Negative	25	6 (23%)	6 (27%)	3 (12%)

IELs, intraepithelial lymphocytes; DQ2, expression of the HLA-DQ2 alleles A1*0501/B1*0201; AGA, anti–gliadin antibodies; TTG, anti–tissue-transglutaminase antibodies.
[a]$P < .01$ vs negative patients.
[b]$P < .05$ vs negative patients.

Gastrointestinal symptom scores and stool frequency at study entry were not different between d-IBS patients with and without HLA-DQ2 or celiac disease–associated IgG. After 6 months of gluten-free diet, the gastrointestinal symptom score in 41 d-IBS patients decreased from 14.5 ± 0.9 (mean ± standard error of the mean) to 10.1 ± 0.9 ($P < .01$). In 20 of 41 (49%) patients, gastrointestinal symptoms improved to scores within the normal range (mean + 2 standard deviations of controls corresponding to 9.3). Stool frequency decreased from 4.1 ± 0.2 to 2.0 ± 0.3 bowel movements/day ($P < .01$) after gluten withdrawal. Fifteen of 41 (37%) patients had formed stools at normal frequency, and in 12 patients both gastroin-

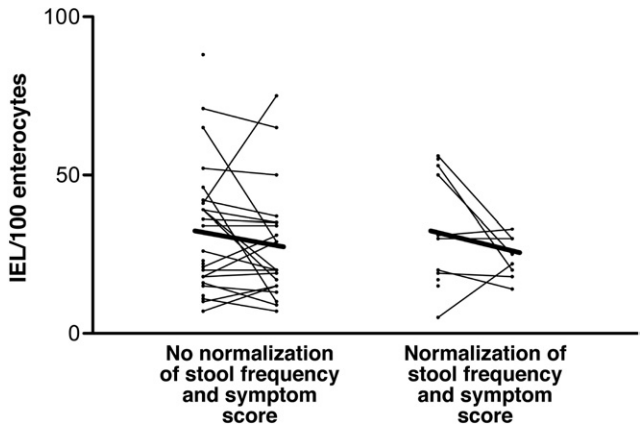

Figure 3. Intraepithelial lymphocytes (*IEL*) in patients with d-IBS before and after gluten-free diet. Duodenal IEL per 100 enterocytes before (n = 41) and after (n = 29) 6 months of gluten-free diet in patients with d-IBS in whom diarrhea and abdominal symptoms resolved (*responders*) and in patients with d-IBS who remained symptomatic (*non-responders*). *Bar* denotes mean changes.

Table 4. Efficacy of Celiac Disease–Associated IgG and HLA-DQ2 Expression in Patients With d-IBS to Predict the Response to Gluten-Free Diet

	Sensitivity	Specificity	Positive predictive value	Negative predictive value
DQ2	92% (61%–100%)[a]	52% (33%–71%)	44% (22%–65%)	94% (70%–100%)
IgG AGA/TTG	75% (43%–95%)	62% (42%–79%)	45% (23%–68%)	86% (64%–97%)
DQ2 and IgG (AGA/TTG)	75% (43%–95%)	76% (56%–90%)	56% (30%–80%)	88% (69%–97%)

DQ2, expression of HLA-DQ2 alleles A1*0501/B1*0201; AGA, anti-gliadin antibodies; TTG, anti–tissue-transglutaminase antibodies.
[a]95% confidence interval.

testinal symptom scores and stool habits returned to normal after 6 months of gluten-free diet.

Although only patients without abnormalities in mucosal architecture were eligible, 11 of the 41 d-IBS patients had increased numbers of intraepithelial lymphocytes. Increased intraepithelial lymphocytes correlated with increased anti-gliadin IgG, but not with HLA-DQ2 expression or increased anti–tissue-transglutaminase IgG (Table 2), or with the response to gluten-free diet (Table 3). There was no difference in intraepithelial lymphocyte counts before or after or in the change of intraepithelial lymphocyte counts after gluten-free diet between responders and nonresponders in 29 patients who had duodenal biopsies after 6 months of gluten-free diet (Figure 3).

A normal gastrointestinal symptom score after gluten-free diet was achieved more frequently in d-IBS patients who were positive for HLA-DQ2 and/or celiac disease–associated IgG antibodies (Table 3). Diarrhea resolved more frequently in HLA-DQ2–positive d-IBS patients with celiac disease–associated IgG antibodies. Higher proportions of patients with both normal stool habits and normal symptom scores after gluten-free diet were found in d-IBS patients expressing HLA-DQ2 and in HLA-DQ2–positive d-IBS patients with celiac disease–associated IgG antibodies (Table 3). The proportion of responders was similar in patients with serum IgG against only gliadin (6/10), only tissue-transglutaminase (1/4), or both antigens (2/6). Sensitivity to predict the response to gluten-free diet was highest (92%) for HLA-DQ2 expression; specificity was highest (76%) for the combination of HLA-DQ2 expression and celiac disease–associated IgG antibodies (Table 4).

Discussion

We recently reported that a subgroup of d-IBS patients identified by the expression of the HLA-DQ2 alleles A1*0501/B1*0201 and increased antibodies against gliadin and/or tissue-transglutaminase in duodenal aspirate might actually have latent/potential celiac disease and could profit from a gluten-free diet.[14] Whereas the expression of HLA-DQ2 can be determined through blood samples, investigating intestinal antibodies is complicated, requiring the often difficult collection of duodenal aspirate and standardization of its binding activity to total Ig concentration. We therefore examined whether serum antibodies against gliadin and/or tissue-transglutaminase can be used as markers of gluten sensitivity.

As expected, increased serum IgA against gliadin and/or tissue-transglutaminase was found in most patients with untreated celiac disease but rarely in patients with treated celiac disease, confirming its significance as a marker for active celiac disease.[23,24] In contrast, increased serum IgG levels against gliadin and/or tissue-transglutaminase were present not only in most patients with untreated celiac disease but also in the majority of patients with treated celiac disease and might therefore represent markers for gluten sensitivity.

In accordance with our earlier findings based on intestinal celiac disease–associated antibodies,[25] approximately one third of d-IBS patients were positive for this putative marker for gluten sensitivity. The proportion of patients with celiac disease–associated serum IgG was significantly higher in d-IBS than in IBD patients, suggesting that abnormal access of antigens to the mucosal immune system through an impaired epithelial barrier is not a major cause for the induction of these antibodies. The link between celiac disease and increased serum IgG levels against gliadin and/or tissue-transglutaminase is supported by the association with HLA-DQ2 expression in d-IBS patients and by the decrease observed in antibody concentrations of HLA-DQ2–positive d-IBS patients receiving a gluten-free diet. Serum IgG antibody concentrations against gliadin did not correlate with IgG antibody concentrations against tissue-transglutaminase, and a considerable proportion of d-IBS patients exhibited increased serum IgG antibodies against only one of the celiac disease–associated proteins. Both parameters are therefore clearly not exchangeable.

Our extended study confirms the improvement of diarrhea in HLA-DQ2–positive d-IBS patients after 6 months of gluten-free diet reported earlier.[14] In addition, a symptom score covering typical gastrointestinal symptoms of IBS patients like abdominal pain or bloating improved to normal values in most patients expressing HLA-DQ2 or with celiac disease–associated serum IgG antibodies after a gluten-free diet. Treatment efficacy in IBS patients is notoriously difficult to prove because of considerable placebo effects,[13,26] and gluten-free diet could improve IBS symptoms unspecifically.[27,28] However, such effects cannot explain the significant association of responses to gluten-free diet with the expression of HLA-DQ2 and celiac disease–associated serum IgG antibodies. In contrast, this association supports the presence of gluten sensitivity in a subgroup of about 17% of d-IBS patients, a proportion remarkably similar to that identified previously by HLA-DQ2 and intestinal celiac disease–associated antibodies.[14]

Furthermore, we found that the determination of HLA-DQ2 expression in combination with serum IgG against gliadin and/or tissue-transglutaminase can predict the response to gluten-free diet in d-IBS patients. Sensitivity to predict the response to gluten-free diet was higher for HLA-DQ2 expression, whereas specificity was higher for celiac disease–associated IgG, and both parameters combined yielded positive and negative predictive values of 56% and 88%, respectively. While not ideal, we consider these values acceptable, because gluten-free diet is a nontoxic and relatively inexpensive treatment, and we used

strict criteria for response, ie, return to normal stool habits and symptom scores.

Rapid clinical remission on a gluten-free diet as seen in our responding patients is one mandatory requirement to diagnose celiac disease according to the revised ESPGAN criteria.[18] The other, villous atrophy in an initial biopsy, was absent by definition in our d-IBS patients. However, it is now recognized that celiac disease not only has highly variable clinical manifestations[7] but also presents a continuum of histopathologic findings,[19] and there is evidence that the sensitivity of IgA–anti–tissue-transglutaminase antibody serologic tests falls sharply with lower degrees of intestinal damage.[29] Clinical and, in most cases, histologic improvement after gluten-free diet has been reported in oligosymptomatic patients with suspected celiac disease who had only Marsh I-II lesions, ie, crypt hyperplasia and/or intraepithelial lymphocytosis.[27] Increased intraepithelial lymphocytes, however, did not correlate with the response to gluten-free diet in our study and were, in fact, absent in the majority of responding patients. Furthermore, changes in intraepithelial lymphocyte numbers were inconsistent and did not correlate with clinical responses after gluten-free diet. Overall histology was therefore not helpful to predict or assess the response to gluten-free diet in our patients.

To our knowledge, most responding patients continue gluten-free diet and remain symptom-free. Several patients reported abdominal discomfort on inadvertent or willful gluten ingestion and were reluctant to formal gluten challenge, which has been recommended to confirm celiac disease in patients with inconclusive biopsy results.[18] Its value, however, in patients with latent/potential celiac disease is unclear. Our experience thus only provides circumstantial evidence of permanent gluten sensitivity, and long-term outcome has not been assessed so far. In addition, there is no evidence that latent/potential celiac disease is associated with complications like T-cell lymphoma. Recommendation of a lifelong strict gluten-free diet to patients with latent/potential celiac disease therefore seems premature at present.

Two recent studies[28,30] indicate that in contrast to other diagnostic procedures, serologic testing for celiac disease might be cost-effective on the basis of an estimated prevalence of about 3%–4% in patients with d-IBS. Our findings, which require confirmation by randomized studies, suggest that screening for HLA-DQ2 and/or celiac disease–associated IgG could identify an additional larger subgroup of patients without villous atrophy or celiac disease–associated IgA who will benefit from gluten-free diet. Our study population in a predominantly referral-based university center who were intensively screened for other disorders might differ from IBS patients who were seen in general practice. In the United Kingdom, celiac disease confirmed by histology has been found in 3.3% and 4.7% of IBS patients in primary[31] and secondary care,[32] respectively, but the prevalence of latent celiac disease is unknown.

Both HLA-DQ2 and celiac disease–associated IgG can be measured from a blood sample, which should facilitate larger studies to determine the prevalence of this condition and the efficacy of gluten-free diet in other settings. Such studies should probably also examine alternative markers of gluten sensitivity because HLA-DQ2 typing is not widely available. In addition, non-diarrheal presentations of celiac disease are increasingly recognized,[7] and the favorable response of d-IBS symptoms apart from diarrhea to gluten-free diet indicates that examination of non-diarrheic IBS patients for gluten sensitivity might be worthwhile.

In conclusion, serum IgG antibodies against gliadin or tissue-transglutaminase in combination with HLA-DQ2 expression are useful markers to identify a subgroup of patients with d-IBS who are likely responders to a gluten-free diet. Whether these patients will develop celiac disease remains to be examined in follow-up studies.

References

1. Green PH, Jabri B. Coeliac disease. Lancet 2003;362:383–391.
2. Alaedini A, Green PH. Narrative review: celiac disease: understanding a complex autoimmune disorder. Ann Intern Med 2005; 142:289–298.
3. Henker J, Losel A, Conrad K, et al. [Prevalence of asymptommatic coeliac disease in children and adults in the Dresden region of Germany]. Dtsch Med Wochenschr 2002;127:1511–1515.
4. Fasano A, Berti I, Gerarduzzi T, et al. Prevalence of celiac disease in at-risk and not-at-risk groups in the United States: a large multicenter study. Arch Intern Med 2003;163:286–292.
5. Maki M, Mustalahti K, Kokkonen J, et al. Prevalence of celiac disease among children in Finland. N Engl J Med 2003;348: 2517–2524.
6. Catassi C, Fanciulli G, D'Appello AR, et al. Antiendomysium versus antigliadin antibodies in screening the general population for coeliac disease. Scand J Gastroenterol 2000;35:732–736.
7. Green PH. The many faces of celiac disease: clinical presentation of celiac disease in the adult population. Gastroenterology 2005; 128:S74–S78.
8. Kagnoff MF. AGA Institute medical position statement on the diagnosis and management of celiac disease. Gastroenterology 2006;131:1977–1980.
9. Rostom A, Murray JA, Kagnoff MF. American Gastroenterological Association (AGA) Institute technical review on the diagnosis and management of celiac disease. Gastroenterology 2006;131:1981–2002.
10. Troncone R, Greco L, Mayer M, et al. Latent and potential coeliac disease. Acta Paediatr Suppl 1996;412:10–14.
11. Ferguson A, Arranz E, O'Mahony S. Clinical and pathological spectrum of coeliac disease: active, silent, latent, potential. Gut 1993;34:150–151.
12. Loft DE, Nwokolo CU, Ciclitira PJ. The diagnosis of gluten sensitivity and coeliac disease: the two are not mutually inclusive. Eur J Gastroenterol Hepatol 1998;10:911–913.
13. Drossman DA, Camilleri M, Mayer EA, et al. AGA technical review on irritable bowel syndrome. Gastroenterology 2002;123:2108–2131.
14. Wahnschaffe U, Ullrich R, Riecken EO, et al. Celiac disease-like abnormalities in a subgroup of patients with irritable bowel syndrome. Gastroenterology 2001;121:1329–1338.
15. Cataldo F, Lio D, Marino V, et al. IgG(1) antiendomysium and IgG antitissue transglutaminase (anti-tTG) antibodies in coeliac patients with selective IgA deficiency: Working Groups on Celiac Disease of SIGEP and Club del Tenue. Gut 2000;47:366–369.
16. Burgin-Wolff A, Gaze H, Hadziselimovic F, et al. Antigliadin and antiendomysium antibody determination for coeliac disease. Arch Dis Child 1991;66:941–947.
17. Thompson WG, Longstreth GF, Drossman DA, et al. Functional bowel disorders and functional abdominal pain. Gut 1999; 45(Suppl 2):II43–II47.
18. Walker-Smith J, Guandalini S, Schmitz J, et al. Revised criteria for diagnosis of coeliac disease: report of Working Group of European Society of Paediatric Gastroenterology and Nutrition. Arch Dis Child 1990;65:909–911.
19. Marsh MN. Gluten, major histocompatibility complex, and the small intestine: a molecular and immunobiologic approach to the

spectrum of gluten sensitivity ('celiac sprue'). Gastroenterology 1992;102:330–354.
20. Rostami K, Kerckhaert J, Tiemessen R, et al. Sensitivity of antiendomysium and antigliadin antibodies in untreated celiac disease: disappointing in clinical practice. Am J Gastroenterol 1999; 94:888–894.
21. Veldhuyzen van Zanten SJ, Tytgat KM, Pollak PT, et al. Can severity of symptoms be used as an outcome measure in trials of non-ulcer dyspepsia and *Helicobacter pylori* associated gastritis? J Clin Epidemiol 1993;46:273–279.
22. Dieterich W, Laag E, Schopper H, et al. Autoantibodies to tissue transglutaminase as predictors of celiac disease. Gastroenterology 1998;115:1317–1321.
23. Sulkanen S, Halttunen T, Laurila K, et al. Tissue transglutaminase autoantibody enzyme-linked immunosorbent assay in detecting celiac disease. Gastroenterology 1998;115:1322–1328.
24. Gomez JC, Selvaggio G, Pizarro B, et al. Value of a screening algorithm for celiac disease using tissue transglutaminase antibodies as first level in a population-based study. Am J Gastroenterol 2002;97:2785–2790.
25. Wahnschaffe U, Stockmann M, Daum S, et al. Intestinal antibodies against gliadin, tissue-transglutaminase, beta-lactoglobulin, and ovalbumin in patients with irritable bowel syndrome. Ann N Y Acad Sci 1998;859:280–284.
26. Cremonini F, Talley NJ. Diagnostic and therapeutic strategies in the irritable bowel syndrome. Minerva Med 2004;95:427–441.
27. Tursi A, Brandimarte G. The symptomatic and histologic response to a gluten-free diet in patients with borderline enteropathy. J Clin Gastroenterol 2003;36:13–17.
28. Mein SM, Ladabaum U. Serological testing for coeliac disease in patients with symptoms of irritable bowel syndrome: a cost-effectiveness analysis. Aliment Pharmacol Ther 2004;19:1199–1210.
29. Tursi A, Brandimarte G, Giorgetti GM. Prevalence of antitissue transglutaminase antibodies in different degrees of intestinal damage in celiac disease. J Clin Gastroenterol 2003;36:219–221.
30. Spiegel BM, DeRosa VP, Gralnek IM, et al. Testing for celiac sprue in irritable bowel syndrome with predominant diarrhea: a cost-effectiveness analysis. Gastroenterology 2004;126:1721–1732.
31. Sanders DS, Patel D, Stephenson TJ, et al. A primary care cross-sectional study of undiagnosed adult coeliac disease. Eur J Gastroenterol Hepatol 2003;15:407–413.
32. Sanders DS, Carter MJ, Hurlstone DP, et al. Association of adult coeliac disease with irritable bowel syndrome: a case-control study in patients fulfilling ROME II criteria referred to secondary care. Lancet 2001;358:1504–1508.

Address requests for reprints to: Dr Ulrich Wahnschaffe, Medical Clinic A, Department of Gastroenterology, Endocrinology and Nutritrion, Ernst Moritz Arndt Universität Greifswald, Friedrich-Loeffler-Strasse 23a; D-17475 Greifswald, Germany. e-mail: ulrich.wahnschaffe@uni-greifswald.de; fax: 49-3834-86-7234.