

Clinical, Serologic, and Histologic Features of Gluten Sensitivity in Children

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Objective To describe the clinical, serologic, and histologic characteristics of children with gluten sensitivity (GS).

Study design We studied 15 children (10 males and 5 females; mean age, 9.6 ± 3.9 years) with GS who were diagnosed based on a clear-cut relationship between wheat consumption and development of symptoms, after excluding celiac disease (CD) and wheat allergy, along with 15 children with active CD (5 males and 10 females; mean age, 9.1 ± 3.1 years) and 15 controls with a functional gastrointestinal disorder (6 males and 9 females; mean age, 8.6 ± 2.7 years). All children underwent CD panel testing (native antigliadin antibodies IgG and IgA, anti-tissue transglutaminase antibody IgA and IgG, and anti-endomysial antibody IgA), hematologic assessment (hemoglobin, iron, ferritin, aspartate aminotransferase, erythrocyte sedimentation rate), HLA typing, and small intestinal biopsy (on a voluntary basis in the children with GS).

Results Abdominal pain was the most prevalent symptom in the children with GS (80%), followed by chronic diarrhea in (73%), tiredness (33%), bloating (26%), limb pain, vomiting, constipation, headache (20%), and failure to thrive (13%). Native antigliadin antibodies IgG was positive in 66% of the children with GS. No differences in nutritional, biochemical, or inflammatory markers were found between the children with GS and controls. HLA-DQ2 was found in 7 children with GS. Histology revealed normal to mildly inflamed mucosa (Marsh stage 0-1) in the children with GS.

Conclusion Our findings support the existence of GS in children across all ages with clinical, serologic, genetic, and histologic features similar to those of adults. (*J Pediatr* 2013; ■: ■-■).

See editorial, p ●●●

Until a few years ago, the spectrum of gluten-related disorders included only celiac disease (CD) and wheat allergy (WA). Recent data, however, suggest the existence of another form of gluten intolerance, known as nonceliac gluten sensitivity, or simply gluten sensitivity (GS).¹

Some individuals experience distress after eating gluten-containing products and show improvement after institution of a gluten-free diet (GFD). Although the gastrointestinal symptoms may resemble those seen in CD, patients with CD do not have positive celiac-related antibodies or intestinal damage. This entity was described more than 30 years ago in 8 adult females suffering from abdominal pain and chronic diarrhea who experienced relief from a GFD and a return of symptoms on a gluten challenge.²

GS is diagnosed in patients with symptoms that respond to removal of gluten from the diet, after CD and WA are excluded. Currently, it is a clinical diagnosis based on response to the GFD and relapse after gluten challenge; no specific blood test is available for GS. Sapone et al,¹ aiming to elucidate the underlying pathophysiological mechanisms of GS, found that GS, as opposed to CD, is a condition associated with prevalent gluten-induced activation of innate, rather than adaptive, immune responses in the absence of detectable changes in mucosal barrier function.

Recently, the existence of GS was confirmed by Biesiekierski et al³ in a double-blind, randomized, placebo-controlled challenge trial performed in a selected group of patients with irritable bowel syndrome who were symptomatically controlled on a GFD. Patients with irritable bowel syndrome-GS frequently demonstrate serum IgG class native antigliadin antibodies (AGA) as a possible marker of immune activation to gluten.⁴

AGA	Antigliadin antibodies
APT	Atopy patch test
CD	Celiac disease
EMA	Endomysial antibody
GFD	Gluten-free diet
GS	Gluten sensitivity
IEL	Intraepithelial lymphocyte
SPT	Skin prick test
tTG	Tissue transglutaminase
WA	Wheat allergy

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The prevalence of GS has not yet been established, although it is estimated that up to 6% of the general population may be affected.⁵ The main problem with this newly identified condition is that a specific biomarker is not yet available to confirm the diagnosis.

No data are available for the pediatric population, although GS may have been diagnosed as a non-IgE-mediated adverse reaction to wheat. We describe a series of children with GS and the serologic genetic and histologic profile that characterized these children, compared with children with CD and healthy gluten-tolerant children, to provide insight into the similarities and differences between these 2 gluten-associated disorders.

Methods

Our case series consists of 15 children with GS (10 males and 5 females; median age, 10.3 years; age range, 1.6-15 years) diagnosed at 2 pediatric gastroenterology centers at the University of Bari and University of Ancona in 2012-2013. These are tertiary referral centers for CD and other pediatric gastrointestinal disorders, each following up more than 800 children with CD. All children were referred to confirm/exclude an adverse food reaction to wheat ingestion. The suspicion of GS emerged after the exclusion of CD and IgE-mediated WA, as recommended by Sapone et al.⁵

All children included in the present series tested negative for: (1) CD serology (IgA endomysial antibodies [EMAs] and IgA tissue transglutaminase [tTG] antibodies); (2) food specific-IgE; (3) skin prick test (SPT) to wheat (extract and fresh food); and (4) atopy patch test (APT) to wheat. To exclude the presence of intestinal disease, a small intestinal biopsy was offered before any dietary intervention. To assess the genetic risk for CD, all children were HLA-typed. After evaluation by a dietitian, all children started (or continued) a GFD for a period of at least 2 months. When the GFD led to rapid disappearance of symptoms, an open gluten challenge of at least 5 g of daily gluten was performed under medical supervision.

The following serologic tests were performed in the children with GS before the gluten elimination diet: (1) CD serologic panel, including tTG IgA and EMA and native AGA IgA and IgG antibodies; and (2) hematologic measurements, including hemoglobin, serum iron, ferritin, aspartate aminotransferase, and erythrocyte sedimentation rate.

For comparison, 15 children with active CD diagnosed according to the European Society of Pediatric Gastroenterology, Hepatology, and Nutrition criteria⁶ and 15 control children enrolled from the children followed in our outpatient clinic for functional disorders⁷ were recruited. Our control group included children with a functional gastrointestinal disorder (according to Rome III criteria) without CD and with no association between symptoms and any particular food. Characteristics of the study patients are summarized in [Table 1](#).

Serologic Testing and Histologic Evaluation

Quantitative detection of native AGA IgA and IgG in human serum or plasma was assessed by indirect solid-phase enzyme

immunoassay (ORGENTEC Diagnostika, Mainz, Germany) test. The cutoff value was set at >10 arbitrary units for both. Quantitative detection of tTG IgA and IgG in human serum or plasma was assessed by an indirect solid-phase enzyme immunoassay test (ORGENTEC Diagnostika). The cutoff value was set at >10 arbitrary units for both. EMA IgA was determined by indirect immunofluorescence using monkey esophagus sections as a substrate (Euroimmun Italia Diagnostica Medica, Padova, Italy). Dilutions >1:10 were considered positive and then titrated. To exclude the presence of selective IgA deficiency (ie, serum IgA concentration <0.05 g/L), serum IgA levels were assayed by nephelometry in all children.

Class II antigen HLA typing (HLA-DRB1* [01, 15, 16, 03, 04, 11, 12, 13, 14, 07, 08, 09, 10], HLA-DRB3*, HLA-DRB4*, HLA-DRB5* and HLA-DQB1* [01, 02, 03]) was done with polymerase chain reaction sequence-specific oligonucleotides using a commercial kit (Diagene, Palermo, Italy).

Small intestinal biopsy was offered to all children with suspected GS while on a gluten-containing diet to assess mucosal status. In accordance with protocol, at least 4 biopsy specimens were obtained, including 1 specimen from the duodenal bulb. Biopsy sections were prepared from duodenal formalin-fixed, paraffin-embedded material; biopsy specimens were histologically evaluated according to the Marsh classification scheme.⁸ Immunostaining to identify intraepithelial lymphocytes (IELs) was performed; intraepithelial lymphocytosis was defined as the presence of >25 IELs/100 epithelial cells.

Allergological Workup

SPT, food specific IgE, and APT were performed to exclude WA. For SPT, wheat extract (Alyostal; Stallergenes, Antony, France) and fresh food (1 g of wheat powder dissolved in 10 mL of isotonic saline solution) were applied to the patient's volar forearm. SPTs were performed using a 1-mm single peak lancet (Allergy Therapeutics, Worthing, United Kingdom), with histamine hydrochloride (10 mg/mL) as a positive control and isotonic saline solution (NaCl 0.9%) as a negative control. Reactions were recorded on the basis of the largest diameter (mm) of the wheal and flare at 15 minutes compared with histamine. The SPT result was considered positive if the wheal was ≥ 3 mm or larger without reaction of the negative control.

Serum samples were analyzed for specific IgE antibody titers against wheat and gluten using a commercially available system (Immuno CAP, Phadia 250; Phadia, Uppsala, Sweden). The cutoff values was set for values > 0.35 kU/L.

APT was performed in all children using fresh food (1 g of wheat powder dissolved in 10 mL of isotonic saline solution) put on filter paper and applied with adhesive tape to the unaffected skin of the back, using 12-mm aluminum cups (Finn chambers on Scanpor tape; Allergopharma, Reinbek, Germany). Isotonic saline solution served as a negative control. The occlusion time was 48 hours, and results were read at 20 minutes and at 24 hours after removal of the cups. At 72 hours after the start of the test, reactions were classified as negative, doubtful (erythema only), weak positive (erythema

Table 1. Clinical and laboratory characteristics of the study subjects

	Controls	GS	CD
Number of children	15	15	15
Age at referral, y, mean $\pm$ SD	8.6 $\pm$ 2.7	9.6 $\pm$ 3.9	9.1 $\pm$ 3.1
Males/females, n	6/9	10/5	5/10
Intestinal symptoms	Abdominal pain, dyspepsia	Abdominal pain, chronic diarrhea, bloating, failure to thrive, vomiting, constipation	Abdominal pain, chronic diarrhea, bloating, failure to thrive
Extraintestinal symptoms	None	Tiredness, headache, limb pain	Anemia, hypertransaminasemia
Celiac serology	All negative	Native AGA-IgG positive in 10, AGA-IgA positive in 1; rest all negative	tTG-IgA and EmA-positive in all
HLA	Not determined	7 DQ2	All DQ2/8
Wheat IgE	All negative	All negative	All negative

and slight infiltration), strong positive (erythema, infiltration, and papules), or very strong positive (erythema, infiltration, papules, and vesicles).

Gluten Challenge

Before the challenge, gluten had been withdrawn from the patient's diet for at least 8 weeks. The challenge was performed in a hospital setting under direct physician observation for 48 hours. The challenge was performed by administering gluten-containing food in increasing doses until a cumulative dose of at least 5 g was reached after 24 hours. This was an open challenge; however, only parents, and not the children, were informed about the gluten administration. Children were monitored throughout the challenge and discharged after 48 hours. Patients were instructed to follow a GFD supplemented with gluten-containing foods provided by the hospital food service for 2 weeks. The children were asked to record all symptoms in a diary provided to them, including: (1) a self-reported visual analog scale (0, no pain, to 10, worst possible pain) and the Faces Pain Scale⁹; (2) a 3-point score of extraintestinal symptoms (0 absent, 1 mild, 2 moderate, 3 severe)⁵; and (3) a gastrointestinal symptoms rating scale.¹⁰ Children with a 30% increase in scores were considered to have a positive challenge.

Statistical Analyses

For continuous variables, the Wilcoxon test was used for comparison of the mean values among children in the 3 groups. The χ^2 test and the 2-tailed Fisher exact test were used, as appropriate, to compare percentages and nominal variables. All statistical tests were 2-tailed and performed at a 5% level of significance. Data were analyzed using SPSS 13.0 (SPSS, Chicago, Illinois).

Results

All children with GS were referred to our attention because of a clear association between wheat consumption and symptoms, along with resolution after GFD. The hospital challenge produced early appearance of symptoms after gluten ingestion (median, 44 hours; range, 38-80 hours). Clinical characteristics of children with GS are summarized in **Table II** (available at www.jpeds.com).

Abdominal pain was the most frequent symptom, reported by 12 children (80%), followed by chronic diarrhea in 11 (73%); fatigue in 5 (33%); bloating in 4 (26%); limb pain, vomiting, constipation, and headache in 3 (20%); and failure to thrive in 2 (13%). Symptoms were always combined except in 2 children with isolated chronic diarrhea and in 1 child with abdominal pain. The most common presentation was a combination of abdominal pain and chronic diarrhea, in 8 children (53%).

Native AGA IgG was the most frequently detected antibody, found in 10 of the 15 children with GS (66%) as well as in 13 of 15 those with CD (86%) and in 2 of 15 controls (13%). Antibody titers were significantly lower in children with GS compared with those with CD (mean level, 21.6 UA/mL vs 87.4 UA/mL; $P < .001$). AGA IgA was found in only 1 child with GS compared with 11 children with CD ($P < .001$). tTG-IgA and EMA antibodies were constantly negative in children with GS. No child had IgA class deficiency.

No difference was found in the determination of nutritional, biochemical and inflammatory markers between GS and controls. Children with CD had significantly lower levels of ferritin and higher levels of liver enzymes. Serologic and hematologic measurements are presented in **Table III**.

HLA typing showed the presence of DQ2 in all children with CD and in 7 of 15 of children with GS (46%). Interestingly, of the 8 DQ2-negative children, 3 had DQ1/7 and three DQ5 or DQ5.

Parents of 11 children with GS (out of 15) provided consent for intestinal biopsy. In these children, histologic analysis revealed a normal to mildly inflamed mucosa (Marsh stage 0 or 1) in the children with GS and subtotal/total villous atrophy with crypt hyperplasia and an altered number of IELs in those with CD (Marsh stage 3a/c). In detail, of the 11 children with GS who underwent the biopsy procedure, 9 (82%) were classified as Marsh 0 and 2 (18%) were classified as Marsh 1. IEL counts were significantly lower in the children with GS compared with those with CD (6.5 ± 1.5 vs 40 ± 16 ; $P < .03$).

Discussion

This series of children with GS supports the existence of this condition in children across all ages with clinical, serologic, genetic, and histologic features partially similar to those reported in adults. GS, a form of gluten intolerance in which

Table III. Serologic and hematologic parameters of children with functional disorders, GS, and CD

	Controls	GS	CD	P
AGA IgG, UA/mL, mean $\pm$ SD	4.5 $\pm$ 4*	21.6 $\pm$ 21.7*	87.4 $\pm$ 81.3	.001
AGA IgG positivity, n (%)	2 (13) [†]	10 (66) [†]	13 (86)	.001
AGA IgA, UA/mL, mean $\pm$ SD	4.1 $\pm$ 2.5 [†]	2.6 $\pm$ 2.7 [†]	46.5 $\pm$ 58.8	.001
AGA IgA positivity, n (%)	0 [†]	1 (6.7) [†]	11 (73)	.001
tTG IgG, UA/mL, mean $\pm$ SD	3.9 $\pm$ 3.6 [†]	2.0 $\pm$ 0.8 [†]	28.5 $\pm$ 28	.001
tTG IgG positivity, n (%)	1 (6.7) [†]	0 [†]	12 (80)	.001
tTG IgA, UA/mL, mean $\pm$ SD	0.6 $\pm$ 0.6*	1.4 $\pm$ 0.7*	137.6 $\pm$ 78.6	.001
Hemoglobin, g/dL, mean $\pm$ SD	12.5 $\pm$ 0.8 [†]	12.8 $\pm$ 1 [†]	12 $\pm$ 1	NS
Iron, ng/mL, mean $\pm$ SD	92.2 $\pm$ 36 [†]	57 $\pm$ 26.2 [†]	69 $\pm$ 32	.05
Ferritin, 15 ng/mL, mean $\pm$ SD	25.8 $\pm$ 6.8 [†]	31.0 $\pm$ 14.8 [†]	15.5 $\pm$ 8.7	.001
Aspartate aminotransferase, U/mL, mean $\pm$ SD	15.9 $\pm$ 4 [†]	18.3 $\pm$ 9 [†]	26.7 $\pm$ 8.8	.005
Erythrocyte sedimentation rate, mm/h, mean $\pm$ SD	7.5 $\pm$ 3.9 [†]	8.0 $\pm$ 2.9 [†]	12.9 $\pm$ 11.5	NS

NS, nonsignificant.

*P < .004.

†P = NS.

neither allergic nor autoimmune mechanisms can be identified, is a recently described disorder that belongs to the spectrum of gluten-related disorders. Whether GS may include patients at the mild end of the celiac spectrum remains to be clarified.⁴ Clinically, GS is characterized by intestinal and extraintestinal symptoms, which occur shortly after ingestion of gluten-containing products and improve rapidly after initiation of a GFD.⁵ These patients differ from those with CD because they do not manifest the typical tTG autoantibodies or other specific celiac-related antibodies and do not sustain intestinal damage. Neither do they show features of an allergic reaction, as shown by lack of IgE or T-cell-mediated abnormalities of immune response to wheat proteins. In the absence of serologic and histologic markers, the diagnosis of GS is still clinical, and it is based on a gluten challenge to assess if the elimination and reintroduction of gluten is followed by the disappearance and reappearance of symptoms.⁵ The pathogenesis of this condition is still debated, although recent data suggest a major role for abnormalities in the innate immune system. The description of this condition is largely restricted to adults, including a large number of patients previously labeled with “irritable bowel syndrome” or “psychosomatic disorder.”²

The children in the present study satisfy the recently proposed criteria for diagnosis of GS.⁵ In all cases, parents observed an apparent association between intake of gluten-containing food and the onset of symptoms, raising suspicion of GS. The clinical presentation was characterized mainly by typical gastrointestinal symptoms, such as abdominal pain and chronic diarrhea. This presentation is similar to that of CD and other functional disorders, and thus the clinical variables cannot be used to address the suspicion of GS. Interestingly, compared with adults, in children the extraintestinal manifestations were less frequent, the most common symptom being fatigue. No child complained of the behavioral changes, “foggy mind,” eczema and/or rash, muscle cramps, or leg numbness typically reported in adults.^{4,11} Unlike in adult patients, in children GS is more common in males than females.

Like adults, children lack a biochemical/serologic/genetic marker of GS. However, the native AGA IgG positivity and

presence of DQ2 were more common (approximately one-half of the cases) in the children with GS compared with controls, a finding in agreement with data from adult GS studies.¹¹ As expected, the children with CD had lower ferritin levels and higher liver function tests values compared with the children with GS and controls. Finally, intestinal biopsy specimens obtained in 11 of the 15 children with GS showed no significant lesions, ruling out the possibility that symptoms might be secondary to other causes of intestinal disorders. An animal model of GS in HLA-DQ8–positive mice provided insight into the mechanisms underlying the relationship between GS, along with functional changes that could explain the development of gastrointestinal symptoms. Indeed, it has been shown that gluten sensitization of DQ8 mice increases acetylcholine release by the myenteric plexus after in vitro nerve stimulation. The increased acetylcholine release is accompanied by hypercontractility and dysmotility, characterized by the increased incidence of retroperistalsis and gastric emptying.¹²

Owing to the lack of a serologic or histologic marker, the diagnosis of GS remains clinical and it is based on a gluten challenge that is usually performed in an open fashion.^{1,11} However, because it is not possible to exclude a placebo effect caused by gluten exclusion/consumption on symptoms in patients with suspected GS, a double-blind, randomized, placebo-controlled gluten challenge should be considered whenever possible.

One of the main challenges for the diagnosis of GS in children is the possible overlap with a delayed allergic reaction to wheat proteins. A significant proportion of infants with an allergic reaction do not have circulating food protein-specific IgE and show negative results to SPTs and serum IgE determinations.¹³ These non-IgE-mediated reactions tend to be delayed, with the onset of symptoms occurring from 1 hour to several days after ingestion of food. Thus, they are often referred to as “delayed hypersensitivity.” The question is whether GS is part of this phenomenon or a different condition. Although there are insufficient data to formulate an answer to this question, some considerations argue in favor of the second hypothesis, as follows: (1) children in our series were older than expected in allergic disorders;

(2) symptoms were not typical for WA (no case of atopic dermatitis or wheat-dependent exercise-induced anaphylaxis); (3) no other allergic manifestation and no family history for atopy was found in our patients; (4) tests (ie, APT) performed to investigate a non-IgE allergic condition were negative; and (5) finally, none of the children with GS had signs of villous derangement or hypereosinophilia in duodenal biopsy specimens.

Whether gluten is the sole responsible agent in GS remains unclear. Gluten is a component of the more complex protein mixture contained in wheat flour, and thus cannot be considered the sole agent responsible for functional symptoms in persons who eat bread and pasta. Other proteins, including alpha-amylase/trypsin inhibitors, and even yeast¹⁴ can contribute to symptoms of GS. Furthermore, whether wheat processing (such as heat treatment) could modify its role in symptom generation is not known. A recent study of 37 adult patients with GS showed a consistent improvement of symptoms with a reduced low-fermentable, poorly-absorbed, short-chain carbohydrate diet, with worsening of symptoms during a gluten or whey challenge.¹⁵ Moreover, *in vitro* studies have suggested that wheat alpha-amylase trypsin inhibitors could play a major role as triggers of the innate immune response in GS.¹⁶

A strength of the present study is the identification of a set of symptoms (abdominal pain and chronic diarrhea) that might be used to identify GS in children. In the past, cases of GS were often misdiagnosed as irritable bowel syndrome.^{4,17} We are aware of the limitations of this study, particularly the small sample size, the refusal of intestinal biopsy in 4 of 15 children, the lack of any estimate of GS prevalence in children, and unavailability of the double-blind placebo controlled food challenge. Although this methodology is considered the gold standard for diagnosing food intolerance disorders, the open challenge is regarded as an acceptable surrogate in current guidelines for GS diagnosis.¹⁸ Because this condition was suspected only in those who complained of gluten-related symptoms, we believe that the reported cases might represent just the tip on a new gluten-related iceberg.

In summary, our study indicates that GS exists in children as well in adults. In children, the clinical picture is dominated by gastrointestinal complaints, a finding that is responsible for a large overlap with functional intestinal diseases. Diagnosis of GS is currently based on a clear-cut association between gluten intake and development of symptoms, after other gluten-related disorders are excluded. More studies are needed to determine the true frequency of GS in children. Until a specific biomarker of this condition is available, the diagnosis of GS should be suspected in selected cases and confirmed by the gluten challenge, given that unnecessary gluten withdrawal may have negative health effects and increased costs for patients and their families.¹⁹ ■

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Table II. Demographic, clinical, serologic, genetic, and histologic data for the 15 children with GS

Patient	Sex	Symptoms	Time to relapse, h	CD Serology	HLA-DQ2/8	Histology
1	Male	Chronic diarrhea	40	All negative	Present	Not available
2	Male	Chronic diarrhea, failure to thrive	38	AGA IgG positive	Absent	Marsh 0
3	Male	Chronic diarrhea	80	AGA-IgG positive	Present	Marsh 0
4	Male	Abdominal pain, chronic diarrhea	38	AGA-IgG/IgA positive	Absent	Marsh 0
5	Female	Abdominal pain, diarrhea/constipation, limb pain	45	All negative	Absent	Not available
6	Male	Abdominal pain, chronic diarrhea, vomiting, headache	44	All negative	Present	Marsh 1
7	Male	Abdominal pain, chronic diarrhea	39	All negative	Absent	Marsh 0
8	Male	Abdominal pain, fatigue, vomiting, headache, limb pain	40	AGA-IgG positive	Absent	Not available
9	Female	Abdominal pain	44	AGA-IgG positive	Present	Marsh 0
10	Female	Abdominal pain, failure to thrive, fatigue, bloating, constipation, vomiting	40	AGA-IgG positive	Present	Marsh 0
11	Male	Abdominal pain, bloating, chronic diarrhea, fatigue	46	AGA-IgG positive	Present	Marsh 0
12	Female	Abdominal pain, chronic diarrhea, bloating, fatigue	47	AGA-IgG positive	Absent	Marsh 1
13	Male	Abdominal pain, constipation	43	All negative	Present	Marsh 0
14	Male	Abdominal pain, bloating, chronic diarrhea, headache	49	AGA-IgG positive	Absent	Not available
15	Female	Abdominal pain, chronic diarrhea, fatigue, limb pain	55	AGA-IgG positive	Absent	Marsh 0