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

Ruggiero Francavilla, MD, PhD[1], Fernanda Cristofori, MD[1], Stefania Castellaneta, MD[2], Carlo Polloni, MD[3], Veronica Albano, MD[4], Stefania Dellatte[5], Flavia Indrio, MD[1], Luciano Cavallo, MD[1], and Carlo Catassi, MD, PhD[4]

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 2014;164:463-7)*.

See editorial, p 436

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 al[3] 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

From the [1]Interdisciplinary Department of Medicine, Pediatric Section, University of Bari; [2]Department of Pediatrics, San Paolo Hospital, Bari, Italy; [3]Department of Pediatrics, Rovereto Hospital, Rovereto, Italy; [4]Department of Pediatrics, Università Politecnica delle Marche, Ancona, Italy; and [5]Tandoi Group, Corato, Italy

C.C. has received consulting fees from Menarini Diagnostics, Schär, and Heinz Italia. The remaining authors declare no conflicts of interest.

 http://dx.doi.org/10.1016/j.jpeds.2013.10.007

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 aminotrasferase, and erythrocyte sedimentation rate.

For comparison, 15 children with active CD diagnosed according to the European Society of Pediatric Gastroenterology, Hepatology, and Nutrition criteria[6] and 15 control children enrolled from the children followed in our outpatient clinic for functional disorders[7] 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 I**.

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