

## Natural History of Potential Celiac Disease in Children

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This article has an accompanying continuing medical education activity on page e36. Learning Objectives—At the end of this activity, the learner will understand the differences between potential and latent celiac disease and the natural history of the former.

See editorial on page 284.

**BACKGROUND & AIMS:** The presence of celiac disease-associated autoantibodies (antiendomysium and antitissue transglutaminase [anti-TG2]) with normal jejunal mucosa indicate potential celiac disease. We performed a prospective, 3-year cohort study to determine the natural history of potential celiac disease in children. **METHODS:** The study included 106 children with potential celiac disease, based on serology analysis and normal duodenal architecture. All but 2 carried the HLA-DQ2 and/or DQ8 haplotype. In all children, every 6 months, growth, nutritional parameters, celiac disease serology, and autoimmunity were investigated. In biopsies,  $\gamma\delta$  intraepithelial-, CD3-, and lamina propria CD25-positive cells were counted; duodenal deposits of anti-TG2 immunoglobulin A were detected. Biopsy analysis was repeated after 2 years on patients with persistent positive serology and/or symptoms. **RESULTS:** Celiac disease was detected primarily in first-degree relatives and patients with autoimmune disorders (40.6%). A gluten-free diet was prescribed to 20/106 patients because of symptoms, which were relieved in only 11. Eighty-nine of the 106 patients entered the follow-up study, with normal daily consumption of gluten. During the follow-up antibodies disappeared in 14.6% and fluctuated in 32.6%. Villous atrophy was observed in 12/39 patients (30.8%) who underwent a repeat biopsy. **CONCLUSIONS: Most children with potential celiac disease remain healthy. After 3 years, approximately 33% of patients develop villous atrophy. Intestinal deposits of anti-TG2 IgA identify children at risk for villous atrophy.**

**Keywords:** Anti-TG2 IgA Intestinal Deposits; Gluten Sensitive Enteropathy; Gluten Free Diet; Anti-TG2 Antibodies.

The diagnosis of celiac disease (CD) is still based on European Society for Gastroenterology Hepatology and Nutrition 1990 criteria: (1) villous atrophy with hyperplasia of the crypts on a normal diet, and (2) a full clinical remission after withdrawal of gluten from the diet.<sup>1</sup> However, nowadays gluten-sensitive enteropathy represents a wide spectrum going from a histologically normal mucosa with only an increased number of intraepithelial lymphocytes (IELs) to a complete

villous atrophy with crypt hyperplasia.<sup>2</sup> In the 1990s Ferguson introduced the terms latent and potential CD.<sup>3</sup> Patients with positive CD-associated antibodies, but a normal, or almost normal, jejunal mucosa are defined as potential celiac patients. Latent CD patients are considered different from potential, as the former had already shown, at least once in their life, severe gluten-dependent villous atrophy.<sup>4</sup> Recently, Matysiak-Budnik et al<sup>5</sup> did a retrospective analysis in a cohort of 61 latent adult CD patients. Patients diagnosed in childhood were subsequently rechallenged and long term latency developed in about 20% of patients who showed normal mucosa without symptoms. As far as potential CD is concerned, anecdotal cases and short series of patients have been published,<sup>6,7</sup> but the natural history of this condition remains unclear.

Markers have been proposed to increase diagnostic sensitivity and specificity and predict the evolution toward overt CD in early celiac enteropathy. An increased density of IEL expressing  $\gamma\delta$  T-cell receptor (TCR),<sup>8</sup> of IELs at the villous tip<sup>9,10</sup> and in particular the detection of antitissue transglutaminase 2 (anti-TG2) immunoglobulin (Ig)A autoantibodies localized below the basement membrane, along the villous and the crypt and around mucosal vessels<sup>11</sup> are considered of great help.<sup>12</sup>

To date the only accepted treatment for CD is a strict adherence to a gluten-free diet (GFD); GFD heals the small intestinal mucosa and normalizes serology. However, there are no clear guidelines on how to deal with potential CD patients, and in particular it is not known whether a lifelong GFD is necessary in asymptomatic patients with normal intestinal mucosa and serum CD-associated antibodies. The aim of this prospective cohort study was to analyze clinical, serological, and histological features of children with positive CD-specific serum antibodies and architecturally normal small intestinal mucosa. In particular, we aimed to define the natural history in the first years after diagnosis and to look for markers predictive of

**Abbreviations used in this paper:** Ad-SoS, amplitude-dependent speed of sound; anti-TG2, antitissue transglutaminase 2; CD, celiac disease; EMA, anti-endomysial antibodies; GFD, gluten-free diet; HLA, human leukocyte antigen; IEL, intraepithelial lymphocyte; Ig, immunoglobulin; TCR, T-cell receptor.

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1542-3565/\$36.00  
doi:10.1016/j.cgh.2010.09.006

the evolution to severe mucosal damage. Our hypothesis, according to the most recent findings,<sup>13</sup> was that the majority of patients develop atrophy in the first 2 years after diagnosis. In fact the main outcome considered has been the evolution to villous atrophy, but also the possible morbidities associated with the condition.

## Patients and Methods

### Patients

The study involved 106 children (74 females, median age 6 years and 8 months; range 18 months–16 years) who underwent a small intestinal biopsy for suspected CD at the Department of Pediatrics in Naples University Hospital Federico II. They were selected according to the presence in their serum of anti-endomysial antibodies (EMA) and/or increased levels of anti-TG2 antibodies and architecturally normal small intestinal mucosa (Marsh 0 and Marsh 1).<sup>14</sup> IgA deficiency was excluded.

### Methods

**EMA and anti-TG2 antibodies.** Serum EMA and anti-TG2 IgA were detected as described<sup>15</sup> by indirect immunofluorescence on frozen section of human umbilical cord as source of antigen and by enzyme-linked immunosorbent assay (ELISA) technique using a kit based on human recombinant antigen (Kit Eu tTg IgA; Eurospital, Trieste, Italy), respectively.

**HLA typing.** The patients were genotyped for human leukocyte antigen (HLA) class II DRB1 and DQB1 molecules. A Dynal Allset+ SSP DR low resolution kit, a Dynal Allset+ SSP DQ low resolution kit, a Dynal Allset+ SSP DQB103, and Dynal Allset+ SSP DQA1 were used for typing.

**Duodenal biopsy and immunohistochemical analysis.** Four distal duodenal biopsies were taken by gastroduodenoscopy from all patients. Three fragments were fixed in 10% formalin, included in paraffin, and then treated for histological and morphometrical analysis. Two experienced pathologists analyzed 4- $\mu$ m thick paraffin hematoxylin stained sections from the 3 fragments by light microscopy and villous height crypt depth ratio (Vh/CrD)  $\geq 2$  was considered normal. This evaluation was made blind to any serology results. One of the duodenal specimens was embedded in an optimal cutting temperature compound (OCT, Tissue-Tek, Sakura), stored at  $-80^{\circ}\text{C}$  and used for immunohistochemical stainings for CD3+, TCR $\gamma\delta$ +, and CD25+ cells as previously reported.<sup>15</sup> The density of cells expressing CD3 and TCR $\gamma\delta$  in the intra-epithelial compartment was determined by counting the number of stained cells per mm epithelium. Cutoff values for CD3+ and TCR $\gamma\delta$ + cells are 34/mm epithelium and 3.4/mm epithelium, respectively. The number of cells expressing CD25 in the lamina propria was evaluated within a total area of 1 mm<sup>2</sup>. Cutoff value for CD25+ cells is 4 mm<sup>2</sup> lamina propria. To determine the cutoff values 100 children with untreated CD and 50 non-CD control children were studied. Percentiles were obtained using the SPSS software. Cutoff values represent the ninetieth percentile of the non-CD patients.

**Intestinal deposits of anti-TG2.** Frozen fragments of small intestinal biopsies were evaluated for the presence of IgA anti-TG2 extracellular deposits. The detection of mucosal anti-TG2 deposits was performed as previously reported.<sup>16</sup>

**Dietary assessment.** The patients' daily gluten intake was compared with that of a control population matched for age and sex. Unaffected sibs and friends of the patients were the control population. Dietary assessment was obtained by a "frequency questionnaire." The amount of daily gluten intake was estimated multiplying the grams of vegetal protein by .8 (standard factor).<sup>17</sup>

**Phalangeal quantitative ultrasound parameters of bone density.** Amplitude-dependent speed of sound (Ad-SoS) measured by phalangeal quantitative ultrasound (DBM Sonic, IGEA, Carpi, Modena, Italy) was used to estimate bone density.<sup>18</sup> The Ad-SoS, m/second and the bone transmission time were measured in the last 4 fingers, the average over the 4 measurements was expressed as Z score, on the basis of the Italian standards provided by the manufacturer.<sup>18</sup> All measurements were performed by the same skilled operator: the coefficient of variations was .8%.<sup>19</sup>

### Study Protocol

At the moment of the first biopsy all patients underwent a clinical (symptoms, growth parameters, daily gluten intake) and laboratory assessment (CD-related autoantibodies, thyroid autoantibodies, nutritional parameters: iron assessment, albumin, prothrombin time); the familial and personal history of autoimmunity was recorded. In a subgroup of patients we analyzed bone density with Ad-SoS. Patients with persistent symptoms were prescribed a gluten free diet and gluten-dependency of their symptoms was determined in the follow-up. The others continued on a normal gluten-containing diet and underwent a 6-months follow-up schedule. After 2 years patients with persistently positive EMA and/or anti-TG2 levels were rebiopsied. The second biopsy was anticipated in the case symptoms ensued.

### Statistics

Data with a Gaussian distribution were compared by the Student *t* test, while Pearson's  $\chi^2$  test was used for nonnormal data. Level of significance was set at  $P < .01$ . Data analysis was performed using SPSS software version 16.0 (SPSS, Chicago, IL).

## Results

### Clinical Features

Forty-three of 106 children (40.6%) in our cohort of potential CD patients were from "at risk groups": 24 were first-degree relatives of celiac patients and 19 were affected by autoimmune diseases (13 type 1 diabetes, 6 thyroiditis). Most of them (86/106; 81.1%) were asymptomatic and accepted to continue a normal diet. Twenty of 106 showed persistent symptoms or conditions which could be attributed to CD; 16 gastrointestinal (recurrent abdominal pain, weight loss, recurrent diarrhea, failure to thrive), and 4 extraintestinal symptoms or conditions (3 short stature, 1 dilatative cardiomyopathy).

### HLA Typing

HLA typing was obtained in 99/106 patients. Ninety-seven of 99 patients had HLA-DQ2 and/or HLA-DQ8 alleles: 70/99 were positive for A1\*0501 and B1\*0201 (DQ2) with the 2 alleles either in cis or in trans, and 12/99 for HLAB1\*0302 haplotype (DQ8); 11/99 patients had both HLA A1\*0501

**Table 1.** Pearson Correlation Between Anti-TG2 Antibodies and Immunohistochemical Parameters at the Moment of the First Observation

	CD3+ cells	$\gamma\delta$ + cells	CD25+ cells	Anti-TG2	Deposits of anti-TG2 IgA
CD3+ cells	1.00	.79 <sup>a</sup>	.48 <sup>a</sup>	.33 <sup>a</sup>	.15
$\gamma\delta$ + cells	.79 <sup>a</sup>	1.00	.44 <sup>a</sup>	.23 <sup>b</sup>	.32 <sup>a</sup>
CD25+ cells	.48 <sup>a</sup>	.44 <sup>a</sup>	1.00	.21 <sup>b</sup>	.12
Anti-TG2	.33 <sup>a</sup>	.23 <sup>b</sup>	.21 <sup>b</sup>	1.00	.36 <sup>a</sup>
Deposits of anti-TG2 IgA	.15	.32 <sup>a</sup>	.12	.36 <sup>a</sup>	1.00

<sup>a</sup>Correlation is significant at the .01 level (2-tailed).

<sup>b</sup>Correlation is significant at the .05 level (2-tailed).

B1\*0201 and HLAB1\*0302 (DQ2 and DQ8), while only 4/99 showed only HLA A1\*0501 or HLA B1\*0201 (half DQ2 heterodimer). Two patients were negative for all the HLA haplotypes associated with CD carrying HLA DQB1\*05/DQB1\*06.

### Dietary Assessment

At the moment of the first biopsy the daily gluten intake of patients with potential CD was 15 grams, with a range from 6 to 40 g/day. These values were similar to those of the control group (data not shown).

### Serology

At the moment of the first biopsy 86/106 patients were positive for both EMA and anti-TG2, 17/106 patients only for EMA associated with borderline anti-TG2 titers and finally 3/106 patients were positive only for anti-TG2.

### Nutritional Parameters

All patients had normal nutritional parameters (iron assessment, albumin, prothrombin time) with the exception of 4 patients who presented low ferritin levels (<10  $\mu\text{g/mL}$ ).

### Assessment of Phalangeal Quantitative Ultrasound Parameters

At the time of the first biopsy 58/106 patients underwent a phalangeal quantitative ultrasound and 3/58 showed Ad-SoS Z score values under 3 SD.

### Histology and Immunohistochemical Analysis

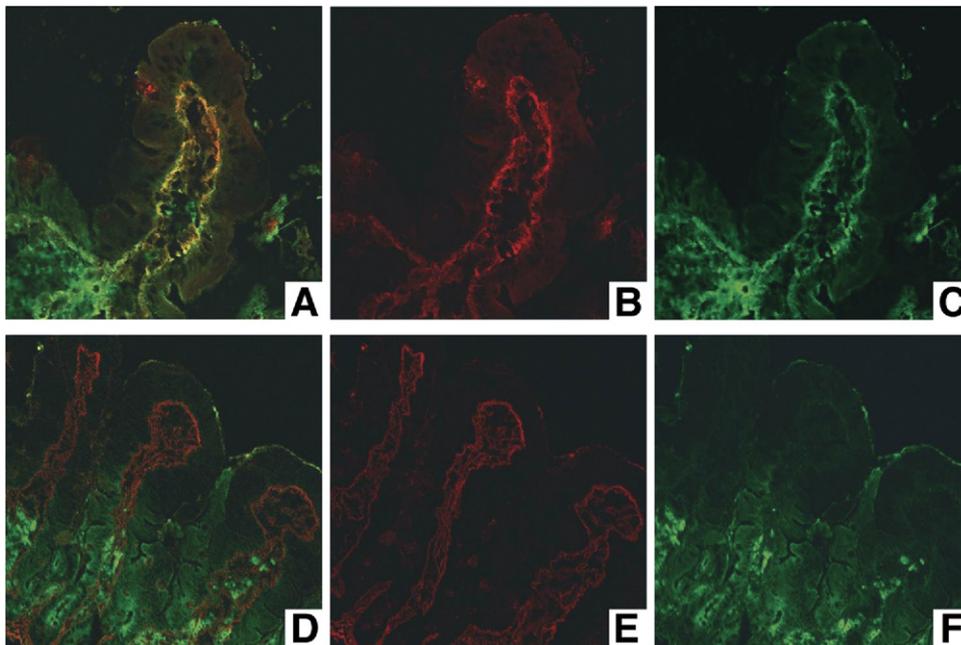
All patients showed a normal architecture of the small intestinal mucosa: 44 patients were Marsh 0 and 62 Marsh 1. Immunohistochemical analysis was performed in all patients. Sixty-two of 106 patients (58.5%) showed an increased number of IEL CD3+ cells (median 37.75; range 7–128) and 76/106 (72.3%) an increased number of CD3  $\gamma\delta$ + cells (median 5.4; range .2–52). In the lamina propria 70/106 patients (66.0%) showed an increased density of CD25+ cells (median 6; range 1–84). We observed a positive correlation between the value of anti-TG2 at the first observation and all immunohistochemical parameters (Table 1). There was also a positive correlation between values of CD3+,  $\gamma\delta$ +, and CD25+ cells with each other (Table 1).

### Intestinal IgA Anti-TG2 Deposits

In 102/106 patients we looked for the presence of intestinal deposits of IgA anti-TG2 and 66/102 (64.7%) were positive (Figure 1). In most of the positive cases a patchy distribution of the deposits was observed with areas of clear positivity and areas with absent signal. The value of anti-TG2 in those patients without deposits (mean  $\pm$  SD: 9.1  $\pm$  6.6 UA) were statistically lower than those of patients positive for intestinal deposits (mean  $\pm$  SD: 31.8  $\pm$  34.2 UA) ( $P < .01$ ). Moreover there was a positive correlation between the presence of deposits and the value of  $\gamma\delta$ + cells (Table 1).

### Follow-Up

**Clinical response in patients that began a GFD.** Twenty of 106 patients began a gluten-free diet because of symptoms, conditions, or signs suggestive of CD: 3 for short



**Figure 1.** (A–C) Detection of IgA deposits in duodenal mucosa from a potential CD patient. TG2 (in red) shows a subepithelial localization (B), IgA in green (C) are present inside plasma cells; thin layers of anti-TG2 antibody mucosal deposits are visible in subepithelial areas. In panel A, yellow color indicates colocalization of IgA anti-TG2 mucosal deposits and TG2. (D–F) Duodenal mucosa from a potential CD patient negative for deposits of IgA anti-TG2. IgA are visible, in green, only inside plasma cells and epithelial cells (F), TG2 in red presents a subepithelial distribution (E). No area of colocalization is evident (D).

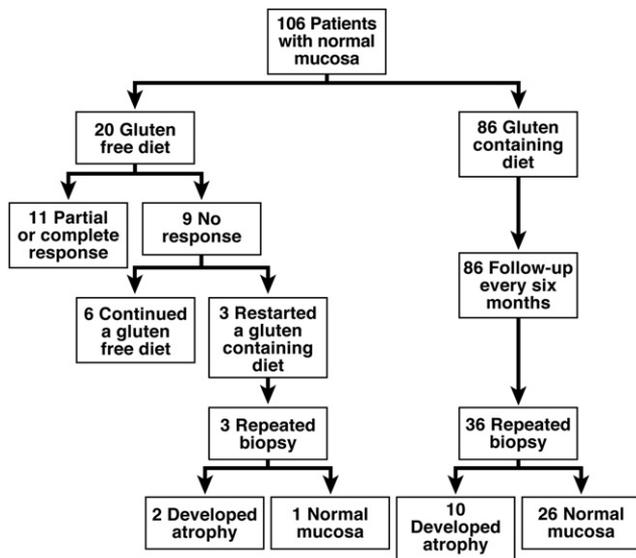


Figure 2. Study population.

stature; 2 for pathologic value of Z score at Ad-SoS; 7 for failure to thrive; 6 for recurrent abdominal pain; 1 for diarrhea; 1 for thyroiditis and dilatative cardiomyopathy. In 1 case with a pathologic value of bone density Z score the parents refused to start GFD. As expected, all patients on a GFD became negative for CD-related autoantibodies. Cardiomyopathy did not change in severity through the follow up period. Nine of 20 did not show any clinical response to GFD (3 short stature; 3 recurrent abdominal pain, and 3 failure to thrive). Thus, in 3 patients a gluten containing diet was resumed (Figure 2).

**Clinical course in patients who continued on a gluten-containing diet.** The 86/106 patients who continued a gluten-containing diet and the 3/20 who resumed a gluten-containing diet after a period of GFD underwent a 6-month follow-up (Figure 2). Persistent gastrointestinal and/or extraintestinal symptoms appeared in 13 patients (6 recurrent abdominal pain, 4 iron deficiency anemia, 2 failure to thrive, 1 constipation).

**Autoimmune Diseases**

During the period of follow up of the 89 patients who continued a gluten-containing diet 1 developed type 1 diabetes a few months after the first biopsy and 3 developed thyroiditis.

**Serology**

Forty-seven of 89 patients (52.9%) who continued a gluten-containing diet had a persistent positive serology for EMA and anti-TG2. In 13/89 (14.6%) serology became completely and persistently negative for both EMA and anti-TG2, while 29/89 (32.6%) showed a fluctuation of antibody titers with transient negativity for EMA or anti-TG2 values. All the patients who showed a fluctuation or negativization of serology presented at the moment of the first evaluation values of anti-TG2 <27 UA; moreover, their titers (mean ± SD: 10.2 ± 6.1 UA) were statistically lower than those of patients who showed a persistent positive serology (mean ± SD: 28.8 ± 34.9 UA) (P < .01).

There was a positive correlation between the first and the last value of anti-TG2 antibodies (Pearson correlation = .578; P < .01)

**Nutritional Parameters**

Four of 89 showed low ferritin and/or anemia as previously mentioned; other nutritional parameters were normal in all subjects.

**Bone Density by Phalangeal Ultrasound**

We repeated the analysis in 21/58 patients on gluten-containing diet and all but 1 were normal after 2 years of follow-up. One of 21 showed a pathologic value of Z score and began a GFD. The patient with a pathologic value at the first evaluation (-3.56) that continued a gluten-containing diet showed a normal value of Z score (-.39 after 2 years of follow-up).

**Second Biopsy**

Thirty-nine of 89 patients who continued on a gluten-containing diet underwent a second biopsy after 2 years of follow-up. The biopsy was performed in 38/39 for positivity for EMA and/or elevated values of anti-TG2 antibodies and in 1 of 39 cases because of onset of abdominal pain, even in the absence of a positive serology. Nine of 39 (23.1%) developed villous atrophy; at the time of the first biopsy 3 of them were Marsh 0 and 6 were Marsh 1. They were diagnosed CD and a GFD was prescribed. In 30/39 the mucosa continued to be normal (10 Marsh 0 and 20 Marsh 1). In 4 of them GFD was anyway prescribed because of symptoms. In all patients with persistently normal intestinal mucosa we performed immunohistochemistry: 20/30 patients (66.6%) showed an increased number of CD3+ cells and 27/30 (90.0%) of CD3 TCR γδ+ cells. No significant increase of CD3 or γδ+ cells or CD25 positive cells was observed between the first and second biopsy.

**Intestinal IgA anti-TG2 deposits.** Thirty-nine of 39 patients that underwent a second biopsy were analyzed for intestinal deposits and we found that 27/39 (69.2%) of them were positive for intestinal deposits and 9/27 showed villous atrophy.

**Third Biopsy**

After 2 years since the second biopsy 6/26 patients that still continued a gluten-containing diet underwent a third biopsy. Three of 6 developed a villous atrophy. Overall 12/39 (30.8%) rebiopsied patients developed villous atrophy.

**Predictive markers of villous atrophy.** To find markers that, at the first observation, could help to identify patients at risk to develop villous atrophy we compared the 12 who developed atrophy with the 27 who did not (Table 2). Only the presence of intestinal deposits in the first biopsy seem to predict the evolution to villous atrophy, in particular patients who did not show deposits had a low probability to develop villous atrophy (Table 2).

**Discussion**

The aim of our work was to evaluate the natural history of children with positive serology for CD, but with a normal intestinal mucosa. According to the definition of Ferguson<sup>3</sup> they are named potential celiac patients. These patients are increasing because of the raised attention for CD and the more

**Table 2.** Predictive Markers of Villous Atrophy

Parameters at the time of first biopsy	CD potential patients who developed villous atrophy (n = 12)	CD potential patients who continued to have normal mucosa (n = 27)	P value
Family history	5 (41.6%)	8 (29.6%)	.46
Autoimmunity	3 (25.0%)	7 (25.9%)	.95
Family history + autoimmunity	8 (66.6%)	15 (55.5%)	.71
Serology persistently positive	9 (75.0%)	14 (51.8%)	.17
Marsh 1	8 (66.6%)	16 (59.2%)	.66
Presence of intestinal deposits of anti-TG2	11 (91.6%)	16 (59.2%)	.04
Increased density of IEL $\gamma\delta+$ cells	10 (83.3%)	19 (70.3%)	.35
Serum anti-TG2 >10 UA/mL	7 (58.3%)	18 (66.6%)	.61

diffused screening of “at risk” subjects. In our institution potential celiac patients now account for nearly 20% of patients with positive serology that undergo a small intestinal biopsy. It is very unlikely that they are false positive subjects, as not only the serology suggests a relation with CD, but also the genetics are consistent with that. All but 2 showed an HLA compatible with CD: this finding is similar to that of a cohort of overt CD cases.<sup>17</sup>

In our patients we can exclude that the absence of damage is related to an insufficient amount of dietary gluten;<sup>17</sup> our data show indeed that these patients had a daily gluten intake similar to that of a control population.

These patients mostly have no symptoms or suffer by light symptoms, often transient, that in many cases resolve even on a gluten-containing diet; on the other hand, in the presence of symptoms suggestive of CD we have considered it unethical not to propose a trial of GFD; however, only a few showed a favorable response to the GFD, thus suggesting in most cases the nongluten dependence of their symptoms.

It is known that undiagnosed CD may predispose to nutritional deficiencies in most cases subtle, also in absence of overt clinical symptoms. This is why we were careful in monitoring the nutritional status of those patients, including a thorough evaluation of the bone status, but no significant problems have emerged from this analysis.

At the moment of the first biopsy in the great majority of patients the anti-TG2 titers were lower than that shown by celiac patients with atrophy, as already shown in a previous work.<sup>15</sup> Some of them became negative during follow-up or showed fluctuant values of anti-TG2 or EMA. These cases were more frequent among those who at the beginning had lower titers of anti-TG2.

All patients were enrolled as they showed a normal intestinal architecture. It should be noted that half of them showed a Marsh 0 lesion, in other terms they did not even show a significant intraepithelial infiltration. However, we noted in the majority of patients signs of T-cell activation with increased  $\gamma\delta+$  cell count.<sup>15</sup> Intestinal deposits of TG2 have been reported to be present at the mucosal level before the appearance of

serum anti-TG2,<sup>11</sup> so that their detection has been proposed to be as the most sensitive and specific test for predicting the diagnosis of CD. Our work showed that 35.3% of potential CD patients are negative for anti-TG2 IgA intestinal deposits, even in the presence of serum EMA and/or anti-TG2. We could not always find concordance between the presence of intestinal deposits and raised serum anti-TG2. We attribute this low sensitivity to the low titers of antibodies in the serum and possibly to their low affinity.

Recently Kurppa et al<sup>13,20</sup> showed by a randomized, controlled study that all the symptomatic patients with mild enteropathy may benefit from a GFD and that all the patients left on a gluten-containing diet developed atrophy after 1 year of observation. Our experience is somewhat different. In contrast with the Finnish studies, we have already mentioned that half of our patients did not show even very mild signs of histological alteration, such as intraepithelial infiltration, but the main difference was that most of them were asymptomatic. The lack of symptoms was accompanied by a completely normal nutritional status. In addition 13/89 (14.6%) become persistently and completely serum negative. These considerations in the absence of data from the literature describing the natural history of these patients has prompted our decision not to prescribe a GFD. On the other hand we cannot disregard that 12/39 (30.8%) developed intestinal atrophy in the short period of follow up and we are very aware that this follow-up should be very strict and prolonged. The only parameters that could help us identify those patients more at risk to develop a more severe damage of the intestinal mucosa seem to be the presence of intestinal deposits of anti-TG2 IgA at the moment of the first observation.

In conclusion, more follow-up is necessary. A random allocation to normal diet or GFD will probably give less biased information on the benefit of GFD in these patients.

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**Conflicts of interest**

The authors disclose no conflicts.

**Funding**

This work was realized with grants from The Regional Network Project for Children and Adolescents affected by Celiac Disease, Campania Region, Italy.