

COELIAC DISEASE

Spectrum of gluten-sensitive enteropathy in first-degree relatives of patients with coeliac disease: clinical relevance of lymphocytic enteritis

M Esteve, M Rosinach, F Fernández-Bañares, C Farré, A Salas, M Alsina, P Vilar, A Abad-Lacruz, M Forné, M Mariné, R Santaolalla, J C Espinós, J M Viver, Barcelona Coeliac Disease Study Group



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Background: Limited data on a short series of patients suggest that lymphocytic enteritis (classically considered as latent coeliac disease) may produce symptoms of malabsorption, although the true prevalence of this situation is unknown. Serological markers of coeliac disease are of little diagnostic value in identifying these patients.

Aims: To evaluate the usefulness of human leucocyte antigen-DQ2 genotyping followed by duodenal biopsy for the detection of gluten-sensitive enteropathy in first-degree relatives of patients with coeliac disease and to assess the clinical relevance of lymphocytic enteritis diagnosed with this screening strategy.

Patients and methods: 221 first-degree relatives of 82 DQ2+ patients with coeliac disease were consecutively included. Duodenal biopsy (for histological examination and tissue transglutaminase antibody assay in culture supernatant) was carried out on all DQ2+ relatives. Clinical features, biochemical parameters and bone mineral density were recorded.

Results: 130 relatives (58.8%) were DQ2+, showing the following histological stages: 64 (49.2%) Marsh 0; 32 (24.6%) Marsh I; 1 (0.8%) Marsh II; 13 (10.0%) Marsh III; 15.4% refused the biopsy. 49 relatives showed gluten sensitive enteropathy, 46 with histological abnormalities and 3 with Marsh 0 but positive tissue transglutaminase antibody in culture supernatant. Only 17 of 221 relatives had positive serological markers. Differences in the diagnostic yield between the proposed strategy and serology were significant (22.2% v 7.2%, $p < 0.001$). Relatives with Marsh I and Marsh II–III were more often symptomatic (56.3% and 53.8%, respectively) than relatives with normal mucosa (21.1%; $p = 0.002$). Marsh I relatives had more severe abdominal pain ($p = 0.006$), severe distension ($p = 0.047$) and anaemia ($p = 0.038$) than those with Marsh 0. The prevalence of abnormal bone mineral density was similar in relatives with Marsh I (37%) and Marsh III (44.4%).

Conclusions: The high number of symptomatic patients with lymphocytic enteritis (Marsh I) supports the need for a strategy based on human leucocyte antigen-DQ2 genotyping followed by duodenal biopsy in relatives of patients with coeliac disease and modifies the current concept that villous atrophy is required to prescribe a gluten-free diet.

See end of article for authors' affiliations

Correspondence to:
M Esteve, Department of Gastroenterology, Hospital Mútua de Terrassa, Universitat de Barcelona, Plaça Dr Robert no 5, 08221 Terrassa, Barcelona, Catalonia, Spain; mestevecomas@telefonica.net

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According to the European Society of Paediatric Gastroenterology and Nutrition criteria,¹ intestinal villous atrophy is a sine qua non for diagnosing coeliac disease and only in this situation should a gluten-free diet (GFD) be recommended. In addition, it was traditionally considered that Marsh I infiltrative lesion (lymphocytic enteritis) was not associated with any symptom or sign of malabsorption.²

However, it has recently been recognised from a short series of selected patients that gluten-sensitive enteropathy (GSE) with preserved villous architecture may be clinically relevant.^{3–6} Anti-endomysial (EmA) and tissue transglutaminase antibodies (t-TGA) are of little diagnostic value in identifying patients with lymphocytic enteritis as they are positive in only 30% of cases.⁷ At present, there is no reliable evidence of how often patients with mild enteropathy have clinical symptoms and of the severity of these symptoms. Consequently, there are no recommendations for the management of patients with mild enteropathy diagnosed in screening programmes for coeliac disease.

First-degree relatives of patients with coeliac disease might benefit from case finding through different diagnostic strategies.⁸ In this sense, about 10% disease prevalence has

been found using serological methods for screening purposes.^{9–10} In our geographical area, specific human leucocyte antigen (HLA)-DQ2 alleles are present in >90% of patients with coeliac disease, 60% of first-degree relatives and 20% of the general population.¹⁰ Thus, DQ2 genotyping identifies susceptible people in a particular risk group.¹¹ To our knowledge, there is no information about the usefulness of HLA genotyping followed by duodenal biopsy in positive cases as a diagnostic strategy of GSE in first-degree relatives of patients with coeliac disease.

The aims of the present study were to evaluate: (1) the usefulness of a strategy consisting of HLA genotyping followed by duodenal histological examination and t-TGA determinations in the culture supernatant of duodenal biopsy specimens from DQ2+ subjects, for the diagnosis of GSE in first-degree relatives of patients with coeliac disease and (2)

Abbreviations: BMD, bone mineral density; EmA, endomysial antibodies; GFD, gluten-free diet; GSE, gluten-sensitive enteropathy; HLA, human leucocyte antigen; IEL, intraepithelial lymphocytes; PCR, polymerase chain reaction; t-TGA, tissue transglutaminase antibodies; VAS, visual analogue scale

the clinical relevance of lymphocytic enteritis diagnosed in this screening programme.

MATERIALS AND METHODS

Subjects

Index cases included both previously and newly diagnosed DQ2+ patients with coeliac disease recruited consecutively in the outpatient clinic of the three participant hospitals between January 2004 and June 2005. All first-degree relatives of every index case were invited to participate in the study, and the family was considered evaluable if at least one first-degree relative agreed to be included. Diagnosis of coeliac disease in index cases was based on the European Society of Paediatric Gastroenterology and Nutrition¹ and American Gastroenterological Association criteria.¹²

Two hundred and seventy six first-degree relatives of 82 DQ2+ patients with coeliac disease (32 males and 50 females; mean age 16.5 years (12 months–77 years)) were identified, and 221 of them (80%; 109 parents, 75 siblings and 37 offspring) were finally included in the study (104 males and 117 females; mean age 34 years (22 months–72 years)). The remaining 55 first-degree relatives (25 parents, 24 siblings and 6 offspring) were not included for several reasons that were recorded in order to disclose a possible selection bias. The reasons for not participating were (1) living in a distant place (50%), (2) being <18 months (9%), and (3) refusal to participate even in the presence of symptoms (41%). For 53 index cases all first-degree relatives could be studied.

Proposed diagnostic strategy and study design

After written informed consent was obtained from the subjects or their parents, blood sampling was performed for HLA-DQ2 genotyping and serum EmA and t-TGA assay. Figure 1 depicts the diagnostic strategy implemented to study first-degree relatives. Duodenal biopsy was carried out on all DQ2+ relatives, irrespective of the results of the serum-specific autoantibody (EmA and t-TGA) assay. t-TGA were also determined in the culture supernatant of duodenal biopsy specimens. First-degree relatives were considered to have GSE if some degree of histological abnormality or a specific humoral response (in serum or duodenal biopsy culture) was found. The presence of anaemia, hypertransaminasaemia, diarrhoea, abdominal pain, abdominal distension, flatulence and asthenia, and serum ferritin concentration were recorded in all DQ2+ relatives. The questionnaire of symptoms was administered to the patients (or parents of paediatric patients). A relative was considered symptomatic when at least one of the above-mentioned symptoms was present. In addition, in adult relatives (n = 67), the following symptoms were quantified by using a visual analogue scale (VAS)¹³ ranging from 0 to 100: diarrhoea, abdominal pain, abdominal distension, flatulence, asthenia, irritability, difficulties in concentration capacity and insomnia. A symptom was considered to be severe if it scored >50 points.

Genetic markers

Standard techniques were used for DNA extraction, polymerase chain reaction (PCR) amplification and product detection. To purify genomic DNA from whole blood, a commercial reagent Generation Capture Column Kit (Gentra Systems, Minneapolis, Minnesota, USA) was used. HLA-DQ2 (DQA1*0501 and DQB1*0201 alleles) genotyping was performed by PCR amplification using sequence-specific primers,¹⁴ on a GeneAmp PCR 2400 System (Perkin-Elmer, Norwalk, Connecticut, USA). PCR products were detected by electrophoresis on a 2% agarose gel and were visualised under ultraviolet light.

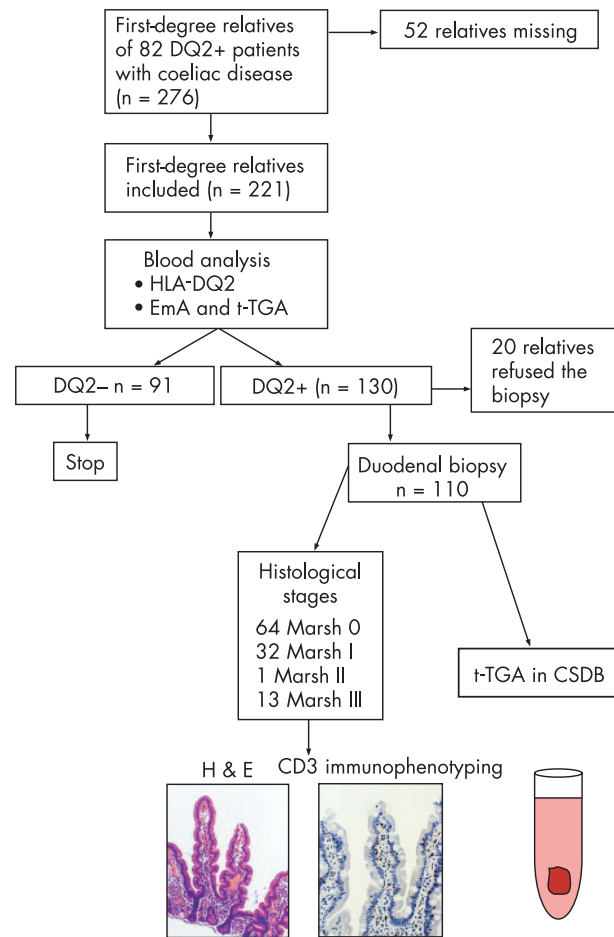


Figure 1 Proposed diagnostic strategy. CSDB, culture supernatant of duodenal biopsy; EmA, endomysial antibodies; HLA, human leucocyte antigen; t-TGA, tissue transglutaminase antibodies.

Duodenal biopsy

Four endoscopic biopsy specimens from the second and third portions of the duodenum were processed using haematoxylin–eosin staining and CD3 immunophenotyping,¹⁵ and they were blindly evaluated by an expert gastrointestinal pathologist (AS). Histopathological findings were staged according to the Marsh criteria,¹⁶ as revised by Rostami *et al*¹⁷: “infiltrative” lesions with intraepithelial lymphocytosis were defined as Marsh I, “infiltrative/hyperplastic” lesions were defined as Marsh II, and “partial (A), subtotal (B) and total (C) villous atrophy” as Marsh III. We assumed intraepithelial lymphocytosis to be present if >25 IEL/100 epithelial cells were observed.¹⁸

Two additional biopsy specimens of the same area were kept for 48 h at 37°C in culture medium (Bio-MPM-1 Multi – purpose SFM for adherent cells (Biological Industries Ltd, Kibbutz Beit Haemek, Israel), antibiotic (100 000 U/ml) and L-glutamine (2 mM)) for t-TGA assay in culture supernatant by using a modification of the method described by Picarelli *et al*¹⁹ (see below). Culture supernatants were collected and stored at –70°C until analysis.

Antibody detection

Serum IgA–EmA was determined by indirect immunofluorescence assay in serum samples at 1:5 dilution, as described previously.²⁰ Commercial sections of monkey distal oesophagus (BioMedical Diagnostics, Marne-la-Vallée, France) were used as indirect immunofluorescence substrate.

IgA-class t-TGA were analysed in the serum and culture supernatant of duodenal biopsy specimens using a quantitative and automated ELISA method by means of a commercially available detection kit (Celikey, Sweden Diagnostics GmbH, Freiburg, Germany) using recombinant human tissue transglutaminase as antigen.²¹ In serum, values >8 U/ml were considered positive, as established by the manufacturer. In culture supernatant, the cut-off value in our laboratory was established by assessing 330 samples of people belonging to risk populations (those with anaemia, first-degree relatives, those with type I diabetes, etc). The IgA-class t-TGA concentration in culture supernatant (1:20 diluted) was analysed using the same calibration curve as in the serum samples (1:100 diluted, as recommended by the manufacturer). Thus, U/ml in culture supernatant were in fact U/ml:5. Results >0.6 U/ml in culture supernatant were considered positive (intra-assay and interassay variability were 5.05 and 8.37, respectively). In the above-mentioned risk population, t-TGA in culture supernatant of duodenal biopsy specimens was positive in 2% Marsh 0, 20.5% Marsh I and 92.4% Marsh III (M Esteve, personal communication, 2005).

Total serum IgA was measured using rate nephelometry (BN II, Dade Behring, Frankfurt, Germany). In cases of IgA deficiency, IgG-class EmA was determined.

Measurement of bone mineral density

Bone mineral density (BMD) was assessed in all DQ2+ relatives having some degree of histological abnormality (Marsh I–III), and in all newly diagnosed cases of coeliac disease (Marsh III), before starting a GFD, recruited in one of the three participant hospitals (Hospital Mútua de Terrassa, Fundació per la Recerca Mútua de Terrassa, Universitat de Barcelona, Terrassa, Catalonia, Spain). BMD was evaluated in 18 patients with Marsh III (12 index cases and 6 relatives; 2 males, 16 females; mean age 35.3 (range 23–77)) and in 26 relatives with Marsh I (11 males, 15 females; mean age 37.1 years (range 12–66)). The t and z scores were measured in lumbar spine and left femoral neck using dual-energy x ray absorptiometry (Lunar DPX-aph, General Electric, Milwaukee, Wisconsin, USA). Osteopenia was defined as a value of BMD >1 SD below the average value of a young adult, but not >2.5 SD below (t score –1 to –2.5). According to World Health Organization criteria, osteoporosis was defined as a value of BMD >2.5 SD below the average value of a young adult (t score <–2.5).²² Comparisons were made to assess differences between people with atrophy (both patients and relatives) and relatives with lymphocytic enteritis.

Statistical analysis

Qualitative parameters were expressed as proportions, whereas quantitative variables were expressed as either mean and standard error of the mean (SEM) or median and range. χ^2 statistics and Fisher's exact test were used to assess considerable associations between qualitative variables. McNemar's test was used to compare paired proportions. One-way ANOVA and Student's t test were used to compare quantitative variables. A p value <0.05 was considered to be significant. All statistical calculations were performed using the SPSS for Windows Statistical package.

RESULTS

Prevalence and histological severity of GSE in first-degree relatives of patients with coeliac disease

Of the 221 first-degree relatives, 130 (58.8%) were DQ2+. In all, 20 (15.4%) DQ2+ relatives with negative serum EmA and t-TGA refused the biopsy. Duodenal biopsy was performed in 110 relatives having the following histological stages: 64 (49.2%) Marsh 0, 32 (24.6%) Marsh I, 1 (0.8%) Marsh II and 13 (10%) Marsh III. The median number of IEL of relatives

with Marsh I lesion was 39% (limits 27–70). Thus, histological abnormalities were found in 46 of 110 relatives who underwent biopsy (41.8%). Figures 2 and 3 provide a detailed description of the histological findings. Table 1 shows the sex and age distribution and the family relationship with the index cases of all DQ2 relatives.

As mentioned previously, for 53 of 82 index cases all family members (89 relatives) could be assessed. Histological findings in these relatives were as follows: 48 (53.9%) Marsh 0, 23 (24.7%) Marsh I, 7 (8.9%) Marsh II–III and 11 (12.4%) did not accept the biopsy. The extent of biopsy acceptance was similar in families totally or partially evaluated (87.7% v 85.6%; p=0.193). There were no significant differences in the degree of histological severity between relatives of families in whom all first-degree relatives could or could not be evaluated (p=0.132).

Relatives with the most severe intestinal damage usually belonged to the same generation as the index case, whereas lymphocytic enteritis was more often found in the parents' generation (fig 2). Consequently, relatives with the most severe lesions (Marsh II–III) were significantly younger (mean (SD) age 24.1(3.7) years) than patients with lymphocytic enteritis (Marsh I; mean (SD) age 32.9 (2.3) years; Student's t test, p=0.044). A non-significant predominance of more severe lesions was observed in females (females: 50.0% Marsh 0, 31.7% Marsh I, 18.3% Marsh II–III; males: 65.3% Marsh 0, 28.6% Marsh I, 6.1% Marsh II–III; χ^2 test, p=0.115).

Diagnostic performance of serology as compared with the present diagnostic strategy

Of the 221 relatives, 16 (7.2%) had positive serological markers (both EmA and t-TGA). The histological spectrum of these patients was as follows: 5 Marsh I, 2 Marsh IIIA, 7 Marsh IIIB and 2 Marsh IIIC. Three relatives with Marsh II, Marsh IIIA and Marsh IIIB, 27 relatives with Marsh I and 64 with Marsh 0 had negative serological markers. The sensitivity of serology for Marsh I and Marsh III detection was 15.6% and 84.6%, respectively.

Of the 221 relatives, 23 (10.5%) had positive t-TGA in the culture supernatant of duodenal biopsy—the 16 relatives with positive serological markers and the other 7 who were negative for serum antibodies (4 Marsh I and 3 Marsh 0). The sensitivity of t-TGA in the culture supernatant in the different histological degrees of GSE was 4.6%, 28.1% and 84.6% in Marsh 0, Marsh I and Marsh III, respectively.

Thus, using the current diagnostic strategy, 49 of 221 relatives (22.2%) showed several degrees of GSE (46 with

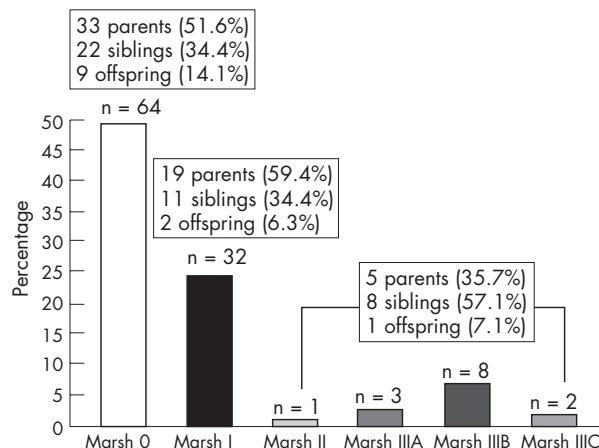


Figure 2 Family relationship in different degrees of histological severity.

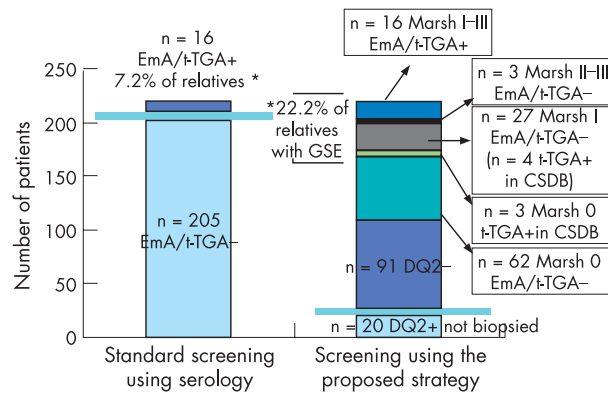


Figure 3 Exploring the coeliac iceberg using the new diagnostic strategy: differences in the diagnostic yield between the proposed diagnostic strategy and standard screening by serology. CSDB, culture supernatant of duodenal biopsy; EmA, endomysial antibodies; GSE, gluten-sensitive enteropathy; t-TGA, tissue transglutaminase.

histological abnormalities and 3 with normal mucosa but positive t-TGA in culture supernatant; fig 3). The difference between the current diagnostic strategy and the standard screening by serology was significant (22.2% v 7.2%, McNemar's test, $p < 0.001$).

Clinical manifestations of GSE in first-degree relatives

Table 2 shows the frequencies of the symptoms related to the degree of histological severity. Relatives with Marsh I and Marsh II-III lesions were significantly more often symptomatic (56.3% and 53.8%, respectively) than patients with normal mucosa (21.1%; Marsh 0; χ^2 test; $p = 0.002$). Flatulence, distension and asthenia were significantly more frequent in Marsh I and II-III than in Marsh 0 ($p = 0.019$, 0.003 and 0.002, respectively). Similar results were obtained when symptoms were assessed only in adult patients, by means of a VAS (table 3). Except for insomnia and difficulties in concentration capacity, a progressive increase in the mean values of the VAS was observed from Marsh 0 to Marsh III (table 3). A significant difference was found for abdominal pain ($p = 0.026$), distension ($p = 0.031$) and asthenia ($p = 0.010$).

Table 4 shows the frequency of severe symptoms (VAS > 50) related to the degree of mucosal lesion. Relatives with Marsh I lesion had more severe abdominal pain ($p = 0.006$) and more severe distension ($p = 0.047$) than relatives with normal mucosa (Marsh 0; Fisher's exact test). Similarly, relatives with Marsh II-III lesion had more severe abdominal pain ($p = 0.005$), more severe distension ($p = 0.024$) and asthenia ($p = 0.024$) than relatives with normal mucosa (Marsh 0; Fisher's exact test).

In all, 4 (6.2%) relatives with Marsh 0, 7 (21.8%) with Marsh I and 3 (21.4%) with Marsh II-III had anaemia. There was a significant difference between Marsh 0 and Marsh I ($p = 0.038$; Fisher's exact test) and a similar, but not significant trend between Marsh 0 and Marsh II-III ($p = 0.104$; Fisher's exact test). Moreover, two of the four relatives with Marsh 0 and anaemia had positive t-TGA (values of 28 and 0.8 U/ml) in culture supernatant of duodenal biopsy specimens. Overall most relatives had normal plasma ferritin concentration. However, a progressive decrease from Marsh 0 to Marsh III was observed (mean (SEM): Marsh 0, 127.5 (22.2) ng/ml; Marsh I, 84.07 (21.27) ng/ml; Marsh II-III, 58.9 (19.26) ng/ml; $p = 0.059$). Similarly, 1 (1.5%) relative with Marsh 0, 3 (9.3%) with Marsh I and 1 (7.1%) with Marsh II-III had hypertransaminasaemia. Differences between Marsh 0 and Marsh I and

Table 1 Sex and age distribution and family relationship between the DQ2+ relatives, relatives who underwent biopsy and relatives with abnormal biopsy

	Family relationship (%)	Sex distribution (%)	Age (median and range)
DQ2+ relatives (n = 130)	65 parents (50.0)	56 males (43.1)	35.0 years (22 months-68 years)
	49 siblings (37.7)	74 females (56.9)	
	16 offspring (12.3)		
Relatives who underwent biopsy (n = 110)	57 parents (51.8)	49 males (44.5)	34.5 years (22 months-68 years)
	41 siblings (37.3)	61 females (55.5)	
	12 offspring (10.9)		
Relatives with abnormal biopsy (n = 46)	24 parents (52.2)	17 males (36.9)	34.0 years (22 months-56 years)
	19 siblings (41.3)	29 females (63.1)	
	3 offspring (6.5)		

between Marsh 0 and Marsh II-III were not significant ($p = 0.113$ and 0.326 , respectively; Fisher's exact test)

Comparison of BMD between patients with lymphocytic enteritis (Marsh I) and those with villous atrophy (Marsh III)

Osteoporosis was diagnosed in only one relative (woman, 72 years) having atrophy (Marsh IIIb). Osteopenia was found in 10 patients with lymphocytic enteritis (37.0%) and in 7 patients with atrophy (38.8%). Taking osteopenia and osteoporosis as a whole, there were no significant differences in the percentage of abnormal BMD between patients with Marsh I (37.0%) and those with Marsh III (44.4%; χ^2 test, $p = 0.761$). There were also no significant differences in mean t and z scores (table 5). There was no significant difference between the age of patients with lymphocytic enteritis (mean (SEM) age: 37.1 (2.1) years) and those with atrophy (mean (SEM) age: 35.3 (2.8) years; Student's t test, $p = 0.615$), whereas a female predominance was found in patients with atrophy (84.6 v 48.4%; χ^2 test: $p = 0.043$).

DISCUSSION

It is accepted that first-degree relatives of patients with coeliac disease may benefit from screening programmes^{23 24} and it is also known that serologic testing (t-TG and EmA) misses a subgroup of patients, particularly those with partial villous atrophy or lymphocytic enteritis.^{7 25} As HLA-DQ2 status is the strongest determinant for coeliac autoimmunity,¹¹ we have

Table 2 Frequencies of the symptoms related to the degree of histological severity

	Normal mucosa (Marsh 0) n = 64 (%)	Lymphocytic enteritis (Marsh I) n = 32 (%)	Architectural distortion (Marsh II-III) n = 14 (%)	p Value
Abdominal pain	15 (23.4)	13 (40.6)	5 (38.5)	0.196
Diarrhoea	14 (21.8)	13 (40.6)	5 (38.5)	0.136
Flatulence	25 (39.0)	22 (68.8)	8 (57.1)	0.019
Distension	14 (21.8)	18 (56.3)	6 (57.1)	0.003
Asthenia	10 (15.6)	15 (46.9)	6 (46.2)	0.002

Statistical comparisons were done by the χ^2 test.

Table 3 Relationship between the values of visual analogue scale and degree of histological severity

	Normal mucosa (Marsh 0) n=32	Lymphocytic enteritis (Marsh I) n=27	Architectural distortion (Marsh II-III) n=8	p Value
Abdominal pain	18.7 (3.0) (0-47)	21.1 (5.6) (0-90)	34.7 (15.7) (0-100)	0.026
Diarrhoea	14.3 (4.3) (0-80)	23.7 (5.3) (0-80)	31.1 (13.5) (0-94)	0.283
Flatulence	37.6 (5.5) (0-90)	42.8 (5.6) (0-100)	46.8 (10.9) (10-90)	0.498
Distension	22.0 (5.6) (0-90)	36.7 (6.5) (0-90)	48.2 (12.8) (0-100)	0.039
Asthenia	15.4 (4.9) (0-80)	31.6 (6.5) (0-100)	52.2 (13.8) (0-100)	0.010
Irritability	18.2 (5.4) (0-100)	27.9 (5.8) (0-100)	44.4 (10.5) (0-100)	0.123
Insomnia	22.8 (5.8) (0-100)	22.9 (5.6) (0-100)	37.5 (12.0) (0-75)	0.443
Difficulties in concentration capacity	23.8 (5.7) (0-100)	27.4 (6.0) (0-100)	23.7 (12.4) (0-90)	0.956

Statistical comparisons were done by one-way ANOVA. Results are expressed as mean (SEM) and range.

used this genetic marker to identify those first-degree relatives of DQ2+ patients who would benefit from histological study. In addition, the presence of specific autoantibodies in culture supernatant of duodenal biopsy specimens was also analysed, on the basis of previous observations showing that EmA detection in these samples may be useful for diagnosing coeliac disease.^{26, 27} Using this diagnostic approach, we have found that 22.2% of first-degree relatives had some evidence of GSE, most of them showing an abnormal histological pattern. This figure is in contrast with the 7.2% that would have been identified using serologic tests alone. It is important to note that the percentage of DQ2+ relatives in the present study (58.8%) was in agreement with those in previous studies.^{10, 11}

The prevalence of coeliac disease diagnosed by means of serum antibody assay in first-degree relatives varied between 2.8%²⁸ and 17.2%²⁹ in different series. Factors accounting for such a high variability have been recently reviewed,³⁰ selection bias (inclusion of families with multiple affected members) probably being the most important one. In the present study, efforts were made to enrol all family members of consecutive adult and paediatric patients with coeliac disease attending the outpatient clinics of the three participant hospitals. The 7.2% prevalence of coeliac disease found in this study using serological testing fits well with previous data from our geographical area.¹⁰ However, the present diagnostic strategy allowed us to identify the whole spectrum

of GSE, including not only patients with atrophy but also patients with lymphocytic enteritis. To our knowledge, this is the first report on the prevalence of lymphocytic enteritis in a particular risk group by using the described diagnostic strategy. In this sense, the 22.2% of cases found in this group provides a reliable estimate of GSE in first-degree relatives of patients with coeliac disease. This concept is further supported by the finding of a similar degree of histological severity in families that were totally or only partially evaluated.

In contrast with previous studies in which in vitro EmA production was analysed,^{19, 26, 27} we have used t-TGA (with similar sensitivity and specificity in serum samples)²⁵ owing to better reproducibility of the ELISA assay. In fact, results identical to those previously reported with EmA were found in patients with atrophy, with 100% positive t-TGA in culture supernatant in patients with positive t-TGA in serum. The assessment of t-TGA in culture supernatant of duodenal biopsy specimens did not significantly increase the diagnostic yield of histological analysis for GSE diagnosis. Only three cases with Marsh 0 and positive t-TGA in culture supernatant were detected. However, two of them had unexplained ferropenic anaemia. Regarding t-TGA positivity in patients with Marsh I, four patients additional to those with positive t-TGA in serum had positive t-TGA in culture supernatant, yielding a 28.1% sensitivity of this assay for lymphocytic enteritis. These are the first data reported in an unselected population of first-degree relatives of patients with coeliac disease. In a previous report, 9 of 12 patients with lymphocytic enteritis and very high lymphocyte count (54-78/100 epithelial cells) had positive EmA in culture supernatant.²⁷ Further studies should clarify whether the assessment of t-TGA in culture supernatant of duodenal biopsy specimens may be a marker for future progression of the disease, identifying those people deserving a closer follow-up.

Recent prospective longitudinal studies^{9, 31} suggest that the progression from lymphocytic enteritis to atrophy would occur in only a small percentage of patients with GSE. In our study, relatives with mild enteropathy were significantly older than patients with atrophy, also supporting this view. Moreover, this finding suggests that most patients with lymphocytic enteritis will probably remain in this phase for a long time, probably representing the most frequent stage of GSE in adults.

In contrast with the previous concept that this type of mild GSE does not produce symptoms,² a similar percentage of both Marsh I and Marsh III relatives were symptomatic as compared with relatives with normal mucosa. It is important to realise that, in the present study, relatives with lymphocytic enteritis were diagnosed by screening and not from symptoms, thus providing the real frequency, not previously reported, of patients with symptoms in this particular group.

Table 4 Frequency of severe symptoms (VAS>50) in different degrees of mucosal lesion

	Normal mucosa (Marsh 0) n=32 (%)	Lymphocytic enteritis (Marsh I) n=27 (%)	p Value*	Architectural distortion (Marsh II-III) n=8 (%)	p Value†
Abdominal pain	0 (0.0)	6 (22.2)	0.006	3 (37.5)	0.005
Diarrhoea	3 (9.3)	5 (18.5)	0.449	3 (37.5)	0.081
Flatulence	11 (33.4)	12 (44.4)	0.592	5 (62.5)	0.229
Distension	6 (18.7)	12 (44.4)	0.047	5 (62.5)	0.024
Asthenia	6 (18.7)	8 (29.6)	0.370	5 (62.5)	0.024
Irritability	6 (18.7)	8 (29.6)	0.370	2 (25.0)	0.649
Insomnia	6 (18.7)	5 (18.5)	1.0	3 (37.5)	0.347
Difficulties in concentration capacity	9 (28.1)	7 (25.9)	1.0	2 (25.0)	1.0

VAS, visual analogue scale.

*Statistical comparisons between normal mucosa and lymphocytic enteritis were done by Fisher's exact test. †Statistical comparisons between normal mucosa and architectural distortion were done by Fisher's exact test.

Table 5 t and z scores of patients with lymphocytic enteritis (Marsh I) and atrophy (Marsh III)

	Lymphocytic enteritis (Marsh I) n = 26	Atrophy (Marsh III) n = 18	p Value
t score lumbar spine	-0.061 (0.22) (-2.45 to 2.08)	-0.641 (0.28) (-3.71 to 1.33)	0.118
t score femoral neck	-0.524 (0.22) (-2.07 to 1.70)	-0.639 (0.19) (-2.03 to 1.09)	0.720
z score lumbar spine	0.153 (0.19) (-1.78 to 2.11)	-0.325 (0.22) (-2.01 to 1.57)	0.122
z score femoral neck	-0.491 (0.21) (-3.13 to 1.69)	-0.467 (0.18) (-1.91 to 1.29)	0.935

Results are expressed as mean (SEM) and range. Statistical comparisons were done by Student's t test.

Abdominal distension and asthenia were the symptoms more consistently associated with GSE, irrespective of the severity of histological lesions. In contrast, insomnia and difficulties in concentration capacity were as frequent in relatives with GSE as in those with normal mucosa, probably indicating that both of these conditions are highly prevalent in the general population and lack discriminant capacity. Interestingly, a progressive increase was found in VAS values related to the progressive impairment of intestinal mucosa. A similar finding was observed for ferritin concentration, suggesting a progressive derangement in nutrient absorption from Marsh I to Marsh III intestinal damage. Nevertheless, similar percentages of anaemia, hypertransaminasaemia and osteopenia were found in patients with Marsh I as compared with those with Marsh III.

As a high percentage of patients with Marsh I were symptomatic, it could be considered that at least these patients might benefit from a GFD. The poorer adherence to a GFD in patients diagnosed from a screening programme as compared with those diagnosed on the basis of symptoms has been argued against case finding in established risk groups or mass-screening programmes.³² However, compliance with a GFD is probably related to the severity of symptoms³³ and, in this sense, a considerably higher proportion of relatives with lymphocytic enteritis than those with normal mucosa had severe abdominal pain and severe distension. The long-term benefit, if any, of preventing complications such as osteoporosis, autoimmune disorders or lymphoma in patients with lymphocytic enteritis is unknown. However, it seems reasonable to advise a GFD at least to those relatives with severe symptoms, loss of bone mass and haematological or biochemical disturbances. It is important to take into account that most of these patients would not have been diagnosed by serology alone. In fact, the need for other diagnostic strategies to improve the detection rate of serology in relatives has been emphasised previously.³⁴

In conclusion, the results of this study modify the current concept of symptomatic GSE and GSE requiring a GFD. In addition, a diagnostic strategy based on HLA-DQ2 genotyping followed by duodenal biopsy in positive relatives seems reasonable, at least in those with symptoms, irrespective of the results of serologic tests.

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Authors' affiliations

M Esteve, M Rosinach, F Fernández-Bañares, M Forné, M Mariné, R Santaolalla, J C Espinós, J M Viver, Department of Gastroenterology, Hospital Mútua de Terrassa, Fundació per la Recerca Mútua de Terrassa, Universitat de Barcelona, Terrassa, Catalonia, Spain
A Salas, Department of Pathology, Hospital Mútua de Terrassa, Fundació per la Recerca Mútua de Terrassa, Universitat de Barcelona, Terrassa, Catalonia, Spain

M Alsina, Department of Immunology (Egarlab), Hospital Mútua de Terrassa, Fundació per la Recerca Mútua de Terrassa, Universitat de Barcelona, Terrassa, Catalonia, Spain

C Farré, Department of Biochemistry, Hospital de Sant Joan de Deu, Esplugues, Catalonia, Spain

P Vilar, Department of Pediatric Gastroenterology, Hospital de Sant Joan de Deu, Esplugues, Catalonia, Spain

A Abad-Lacruz, Department of Gastroenterology, Hospital Sant Llorenç de Viladecans, Viladecans, Catalonia, Spain

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Other members of the Barcelona Coeliac Disease Study Group are Miquel Carreras, Roger Garcia, Department of Pediatrics, Hospital Mútua de Terrassa, Fundació per la Recerca Mútua de Terrassa, Universitat de Barcelona, Terrassa, Catalonia, Spain; Jaume Casalots, Clara Isabel González-Minguez, Department of Pathology, Hospital Universitari Mútua de Terrassa, Fundació per la Recerca Mútua de Terrassa, Universitat de Barcelona, Terrassa, Catalonia, Spain; Elena Monzón, Department of Internal Medicine, Hospital Mútua de Terrassa, Fundació per la Recerca Mútua de Terrassa, Universitat de Barcelona, Terrassa, Catalonia, Spain; Luís del Río, Department of Nuclear Medicine, CTD-CTIR, Barcelona, Catalonia, Spain; Victòria Cusi, Department of Pathology, Hospital Sant Joan de Deu, Esplugues, Catalonia, Spain; Mercè Barenys, Department of Gastroenterology, Hospital Sant Llorenç de Viladecans, Viladecans, Catalonia, Spain.

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M Esteve, M Rosinach, F Fernández-Bañares, et al.

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