Increased prevalence of celiac disease in first and second-grade relatives. A report of a family with 19 studied members

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RESUMEN

Introducción: la enfermedad celiaca (EC) es un proceso autoinmune, desencadenado por la ingesta del gluten contenido en la mayor parte de los cereales, que afecta a individuos genéticamente predispostos. Por todo ello, muestra una clara tendencia familiar, centrada fundamentalmente en marcadores del sistema HLA de clase II.

Objetivos: nos propusimos en el presente trabajo analizar la prevalencia de EC en una familia extensa, a partir de un caso índice fallecido hacía unos años, como consecuencia de padecer la misma enfermedad, complicada además con el desarrollo de un tumor maligno del intestino delgado, del tipo del adenocarcinoma.

Métodos: se estudiaron un total de 19 miembros. Se les realizó un protocolo diagnóstico que incluía una historia clínica detallada, junto con una hemograma y estudio de coagulación, una bioquímica amplia incluyendo pruebas de función hepática, estudio sérico del metabolismo del hierro, niveles circulantes de ácido fólico y vitamina B12, pruebas de función tiroidea, determinación de la transglutaminasa tisular y marcadores genéticos (DQ2 y DQ8). En los casos sospechosos y para su confirmación se realizó gastroscopia completada con toma de biopsias duodenales múltiples.

Resultados: encontramos una prevalencia global de EC en 9/19 de los familiares estudados, lo que representa un 47,4%, distribuidos de la siguiente manera en función del parentesco con el caso índice: cuatro de siete hermanos (57%); uno de tres hijos (33,3%); tres de ocho sobrinos (37,5%) y el único sobrino-nieto estudiado de nueve años de edad, también estaba afecto.

Conclusiones: de todo ello se deduce la necesidad de hacer estudios amplios familiares, cada vez que se diagnostica un paciente de enfermedad celiaca, incluyendo familiares de primero y segundo grado, dada la relativa facilidad actual para llevarlos a cabo y la elevada prevalencia encontrada.


ABSTRACT

Introduction: celiac disease (CD) is an autoimmune condition that is triggered by the ingestion of gluten, a substance present in most cereals, and that affects genetically predisposed individuals. As a result, this condition is clearly familial, and mainly associated with HLA class II markers.

Objectives: in this work we set out to analyze the prevalence of CD in an extensive family based on an index subject who had already died from this disease a few years ago, where CD had been complicated by the development of a small-bowel malignancy, namely an adenocarcinoma.

Methods: nineteen members were studied. They all were subjected to a diagnostic protocol including a detailed medical history, hemogram, coagulation tests, and blood biochemistry (including liver function tests, serum iron metabolism, circulating folic acid and vitamin B12 levels, thyroid function tests, tissue transglutaminase measurement, and genetic markers (DQ2 and DQ8). Suspect cases underwent gastroscopy plus multiple duodenal biopsy for confirmation.

Results: overall we encountered CD in 9/19 studied members, which represents 47.4% with the following distribution according to degree of kinship –four of seven siblings (57%); one of three children (33.3%); three of eight nephews and nieces (37.5%), and the only grandnephew, who was 9 years old.

Conclusions: from all this it may be seen that family studies are needed every time a patient is diagnosed with celiac disease; these studies should include both first- and second-degree relatives, given the high prevalence encountered and the fact that these tests are relatively straightforward to perform.

Key words: Celiac disease. Family study. Clinical-serological screening. First- and second-degree relatives.
INTRODUCTION

Celiac disease (CD) is an autoimmune condition primarily affecting the gut, most particularly the small bowel, and characterized by the presence of chronic inflammation at the duodenal mucosa; it is commonly—but not always—associated with the presence of atrophied intestinal villi, and on occasion manifests with malabsorption and a range of diverse clinical complaints that may develop during childhood or at any other age. It is currently recognized as a common disease, but still remains poorly understood by many physicians and clearly underdiagnosed in adults (1-3).

It is triggered by the ingestion of gluten and related proteins, which are exclusively present in wheat, rye, and barley. It affects genetically predisposed individuals who carry the antigen DQ2, which is expressed in 95% of patients, with the rest being DQ8 (+). Removal of gluten-containing foods from the diet leads to permanent clinical and histological improvement (4,5).

There is strong genetic susceptibility in the development of CD, as confirmed by the high consistency seen in monozygous twins (up to 75%) (6).

There is a high prevalence of CD in first- and second-degree relatives, which is around 4-12% as previously described (7). This relationship is mostly due to the presence of relevant genetic grounds conditioned by the presence of the aforementioned HLA-II antigens (DQ2 and DQ8).

Prior studies have confirmed an increased risk of various CD-associated gastrointestinal and extra-gastrointestinal tumors, which usually develop in patients diagnosed in adulthood. However, the presence of intestinal lymphoma must still be considered a rare complication in this disease (8,9).

PATIENTS AND METHODS

A family-based study of celiac disease was performed on an outpatient basis at CD Clinic, Gastroenterology Department, Hospital Central de Asturias in Oviedo, and all first-degree relatives of an index case patient who died 15 years ago from celiac disease with an associated small-bowel malignancy were invited.

In all, 19 family members voluntarily entered the study, and were distributed as follows: six siblings, three children, eight nephews and nieces, and a grandnephew.

All relatives studied had a detailed medical history obtained, and underwent a complete physical exploration and laboratory tests, including CBC, coagulation, liver function, thyroid function, iron metabolism (sideremia, ferritin, transferrin saturation), and serum folic acid and vitamin B12 levels.

Regarding CD serology only antibodies against IgA-class tissue transglutaminase (TTG) type 2 were measured using a commercially-available ELISA (Orgentec, Diagnostika GmbH, Mainz, Germany, which uses a human recombinant TTG extracted from an embryony kidney cell line—293-EBNA). Sera underwent several dilutions using Tween 20 (TET), and incubation with human TTG for 1.5 hours at room temperature. Color absorbances obtained were read using a colorimeter at 450 nm wave length. Values above 1 U/mL were considered positive (10).

To examine genetic susceptibility markers 2 mL of peripheral blood were drawn and then mixed with 2 mL of absolute alcohol, the whole being stored until sample analysis. During the test alcohol was removed by washing extracts twice with phosphate buffer solution (PBS), and samples obtained were analyzed for HLA-DQ2 measurement using the PCR-SSP (specific sequence of primers) technique; negative samples were tested for DQ8 using the same procedure, according to a previously-described method (11).

In cases with a clinically- and laboratory-based suspicion of celiac disease an upper digestive endoscopic exam was performed, and multiple duodenal biopsies were collected (at least 4) using a Fujinon fiberduodenoscope, model EG-250WR. The morphological study of biopsies was carried out in paraffin-embedded sections with standard hematoxilin/eosin stain. Intraepithelial lymphocyte counts were performed with CD-8-specific immunohistochemistry techniques, and were considered positive when their density was equal to or higher than 40 per every 100 enterocytes. Mucosal atrophy extent (partial, subtotal, total) was expressed as the remaining CD-related histological findings, including crypt hyperplasia and presence of inflammatory infiltrates in the lamina propria, that is, by using Marsh’s classification, which dates back to 1990 (12).

All relatives with compatible manifestations and lab tests, plus positive genetic markers, increased TTG, and consistent histology were diagnosed with CD.

RESULTS

Among all 19 family members studied we found 9 patients with celiac disease, which represents a very high prevalence around 47.4%, with the following distribution: 4/7 siblings (57%); 1/3 children (33.3%); 3/8 nephews and nieces (37.5%), and 1/1 grandnephews.

The family tree of all studied relatives is illustrated, including healthy and CD individuals. The parents and two siblings of the index patient could not be examined, as they had already passed away several years prior to the present study (Fig. 1).

The clinical, genetic, serologic, and histological characteristics of involved relatives are pooled in a detailed and comparative manner (Table I).
Following is a detailed description of both the index patient and his affected relatives:

**Index patient (case 1)**

JeRB. A 37-year-old, married male with three children, one of them with CD (case 5). He was diagnosed with celiac disease in 1974, when he was 30, based on clinical and laboratory criteria; the diagnosis was confirmed by intestinal biopsy, which showed severely atrophied intestinal villi (Marsh’s stage 3c). DQs were not determined. He began a gluten-free diet whereby he experimented clinical improvement—he regained 8 kg of body weight, and lost sight of previous diarrhea. He remained symptom-free until 1981, a time when he experienced severe abdominal pain with subocclusive attacks and relevant general impairment in association with severe weight loss. Exploration revealed a tumor in the proximal (duodenal-jejunal) small intestine, accompanied by liver metastases; a biopsy of the latter revealed a little-differentiated adenocarcinoma, and the patient died two months later.

**Case 2**

HRB. A 70-year-old male, the brother of the index patient, married, with three healthy children. A 5-year-old grandson of his is a hemophiliac. He was operated on for nasal polyps in association with allergic rhinitis on three occasions. He was diagnosed with CD 2 years ago based on his 20 years’ standing diarrhea; he has been on GFD ever since, with a good clinical response—diarrhea remitted and the patient gained 9 kg of body weight. No associated skin involvement. At the time of diagnosis his lab tests were within normal range, with increased TTG (93.2 U/mL). He is DQ2 (+), and a duodenal biopsy revealed mildly atrophied duodenal villi (Marsh’s stage 3A).

**Case 3**

MRB. A 67-year-old male, brother to the index patient. Married, with two children, one of them diagnosed with multiple sclerosis. Repeat rhinitis and pharyngitis since childhood. Generalized psoriatic lesions in elbows, knees, and scalp for 25 years. Blood tests showed sustained leukopenia (3,700 cells) associated with slightly decreased ferritin (21 ng/mL) with no anemia and decreased serum vitamin B12 (177 pg/mL). TTG was mildly elevated (1.74 U/mL) (N ≤ 1). The patient is DQ2 (+), and his duodenal biopsy was considered normal (Marsh’s stage 0). He was diagnosed with CD 6 years ago, and put on GFD, with good clinical and laboratory response.

**Case 4**

JoRB. A 57-year-old woman, sister to the index patient. Married, with a 1 daughter with CD who is 35 years old (case 6). She had three miscarriages (one before and

### Table I. Clinical-laboratory characteristics for the index patient and relatives with CD

<table>
<thead>
<tr>
<th>Rel./case</th>
<th>Age/Gender</th>
<th>Manifestations</th>
<th>TTG (U/mL)</th>
<th>DQ2</th>
<th>Duodenal biopsy (Marsh)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Index case (II-8)</td>
<td>37/M</td>
<td>Gen. syndrome</td>
<td>–</td>
<td>–</td>
<td>ileal adenocarcia</td>
</tr>
<tr>
<td>Brother (II-1)</td>
<td>70/M</td>
<td>Diarrhea</td>
<td>93.2 (+)</td>
<td>(+)</td>
<td>3a</td>
</tr>
<tr>
<td>Brother (II-3)</td>
<td>67/M</td>
<td>Psoriasis</td>
<td>1.7 (+)</td>
<td>(+)</td>
<td>0</td>
</tr>
<tr>
<td>Sister (II-6)</td>
<td>57/M</td>
<td>Asymptomatic</td>
<td>1.5 (+)</td>
<td>(+)</td>
<td>2</td>
</tr>
<tr>
<td>Son (III-10)</td>
<td>29/M</td>
<td>Altered LFTs</td>
<td>2.4 (-)</td>
<td>(-)</td>
<td>3b</td>
</tr>
<tr>
<td>Niece (III-4)</td>
<td>42/F</td>
<td>Hypothyroidism HD</td>
<td>1.8 (-)</td>
<td>(-)</td>
<td>2</td>
</tr>
<tr>
<td>Niece (III-6)</td>
<td>35/F</td>
<td>Constipation</td>
<td>1.9 (-)</td>
<td>(-)</td>
<td>1</td>
</tr>
<tr>
<td>Nephew (III-7)</td>
<td>17/M</td>
<td>HD</td>
<td>0.8 (-)</td>
<td>(-)</td>
<td>0</td>
</tr>
<tr>
<td>Grandnephew (IV-1)</td>
<td>9/M</td>
<td>HD</td>
<td>0.9 (+)</td>
<td>(+)</td>
<td>1</td>
</tr>
</tbody>
</table>

Rel.: relation; M: male; F: female; HD: herpetiform dermatitis.

Case identification is related to its representation in the family tree.
two after her daughter’s birth). Associated allergic rhinitis. No gastrointestinal complaints. Blood tests showed sustained leukopenia (4,450 cells) with no associated anemia. She was transiently positive for smooth muscle antibodies (SMAs) (medium titers: 1/320) after a measurement performed 2 years ago, but such antibodies were cleared with no recourse to immunosuppressing therapy; LFTs were normal. TTG was mildly elevated (1.5 U/mL). The patient is DQ2 (+), and her duodenal biopsy showed the presence of dense chronic inflammatory infiltrates in the lamina propria, with no atrophied villi (Marsh’s stage 2). She has been on GFD for one year now, and response is good.

Case 5
CSR. A 42-year-old niece to the index patient. Married, with a celiac son (case 9). Her mother, CRB, who is 65 years and sister to the index case, does not meet CD’s clinical or laboratory criteria, but has persistently positive anti-thyroid antibodies in high titers, with normal thyroid functioning. She has chronic rhinitis and epilepsy since childhood, and has enamel maturation defects as well as recurrent skin lesions diagnosed as herpetiform dermatitis (HD). Frequent abdominal pain since youth, with normal bowel habit. She also has hypothyroidism, and is on replacement therapy with Levothroid-50 mcg (1 tablet/day). Moderate, persistent thrombopenia (142,000) with no associated leukopenia or anemia was documented. TTG has been mildly positive (1.8 U/mL). The patient is DQ2 (-) and DQ8 (+). Duodenal biopsies show the presence of relevant chronic inflammation at the lamina propria (Marsh’s stage 2).

Case 6
FGR. A 35-year-old niece to the index case. She is the daughter of JoRB (case 4). Single. She was born at 8 months after dystocic labor with her umbilical cord around her neck. As a result, she has brain palsy with significant mental and psychomotor retardation, has difficulty walking on crutches, and partly uses a wheelchair. She usually experiences bloating, and meals do not agree with her. Common epigastric heartburn in association with reflux. Severe constipation usually needing laxatives. Blood tests show persistently decreased ferritin (6 ng/mL) and TSI (13.4%) with no anemia. Anti-thyroid antibodies are mildly positive (163 U/mL), and thyroid functioning is normal. TTG is slightly positive (1.9), DQ2 is negative, DQ8 is positive, and duodenal biopsies showed clearly increased intraepithelial lymphocytes (Marsh’s stage 1).

Case 7
JRA. A 16-year-old nephew. No relevant history. No symptoms. Generalized, highly pruriginous skin rash, particularly in the face, arms, and legs, since childhood, diagnosed as herpetiform dermatitis (HD). Blood tests show mild leukopenia (4,800), and decreased total cholesterol (116 mg/dL). TTG was normal (0.88 U/mL). He is DQ2 (-) and DQ8 (+). Duodenal biopsies were normal (Marsh’s stage 0). He was put on GFD 10 months ago, with good clinical and laboratory response; skin lesions were cleared.

Case 8
CJRM. A 29-year-old male. Middle of three siblings (the remaining two are healthy). Married, with no children. Non-drinker, non-smoker. Repeat tonsilitis during childhood, he was operated on at 6 years of age. Asymptomatic. Normal bowel habit. Normal lab tests, with mildly, persistently elevated transaminases. Positive TTG (2.46). DQ2 (-) and DQ8 (+). Duodenal biopsy showed moderately atrophied intestinal villi, associated with significant inflammatory infiltration in the lamina propria (Marsh’s stage 3b). He has been on GFD for 2 years now, with good clinical and laboratory responses.

Case 9
OVS. A 9-year-old grandnephew to the index case. The son of CRS (case 5). Repeat allergic rhinitis since 1 year of age; recurrent pruriginous skin rash consistent with HD. No gastrointestinal complaints. Normal bowel habit with a slight trend towards constipation. Normal lab tests. TTG within normal range (0.9). He is DQ2 (+). His duodenal biopsy showed minimal changes (Marsh’s stage 1). He has been on GFD for 1 year now, with a good response.

DISCUSSION
CD is a chronic intestinal disease caused by permanent intolerance to gluten. The small bowel has a considerable functional reserve, which explains why many affected individuals exhibit few or no symptoms, many of them with no associated malabsorption.

Clinical presentation depends on various factors such as age at onset, extent of gluten susceptibility, and amount of cereal flour used. Atypical, extra-intestinal manifestations are variable and potentially related to the various responses of organs and tissues to gluten (13).
A number of epidemiological studies performed in several geographic regions during the past few years revealed a very high number of non-diagnosed cases among the general population, possibly tenfold higher than that of currently diagnosed patients (14).

Cumulative prevalence is high in so-called “at-risk groups” for CD, including patients with chronic enteropathic anemia refractory to oral replacement therapy, osteoporosis, delayed growth, infertility, associated autoimmune conditions, and a family history, among others (15).

Diarrhea occurs in fewer than 50% of patients at the time of presentation, versus almost 100% of patients diagnosed during the 1960s. Weight loss is currently uncommon; when present, it usually means a severe presentation form with extensive, significant intestinal involvement. In contrast, up to 30% of patients are overweight at the time of diagnosis (16,17).

Symptom onset is usually gradual, and a diagnosis is reached several years later. On occasion patients report a symptom-triggering process, including acute gastro-enteritis (AGE) episodes, a trip abroad (particularly to developing or tropical countries), stress, or any surgical procedure.

 Constitutional symptoms such as astenia, decreased appetite, and depression are commonly seen, but do not suffice regarding diagnostic suspicion.

Pain, abdominal bloating, and bowel habit changes develop in the absence of malabsorption, and this set of complaints is usually identical to that of patients with irritable bowel syndrome (IBS). According to Rome criteria, the latter may have celiac disease in a high percentage, usually above 5%, and hence should routinely undergo serologic tests for TTG measurement, in order to exclude or confirm this diagnosis (18).

Iron and folic acid deficiency is common, separately or combined, and anemia may be associated. Vitamin B12 deficiency is rarely seen, as its absorption is cofactor-dependent and occurs in the terminal ileum, usually unaffected by CD.

Interleukin 15 (IL-15) has been recently seen to be highly increased in the intestinal mucosa of celiac patients. This cytokine is expressed by innate immune system cells such as enterocytes and monocytes in the lamina propria. This points out the relevance of this system in the early pathogenesis of this disease, and suggests the potential presence of a direct toxic mechanism of gluten on the intestinal epithelium, in association with damage induced by the acquired immune system as mediated by CD-8 lymphocytes and other pro-inflammatory cytokines such as TNF-alpha (19).

The most important point for diagnosing a patient with celiac disease is to bear this condition in mind in all patients with frequent gastrointestinal complaints whether associated with systemic manifestations or otherwise. No single procedure can definitely diagnose or rule out CD in a given individual. It is the combination of clinical, laboratory, genetic, and histologic data what usually allows a definitive diagnosis; in doubtful cases, though, prescribing a gluten-free diet (GFD) for at least six months, and monitor the patient’s response may be of help (20).

A range of duodenal endoscopic changes has been described for celiac patients, including mucosal pallor, a “mosaic pattern”, festooned folds, etc. Such changes are to a certain extent correlated to histologic changes, but very often are absent; the duodenum must then be systematically biopsied with 4-6 collections—particularly when exploring patients at risk—to try and increase the number of diagnoses (21,22).

Most typical histologic findings include flattened or atrophied villi, crypt hyperplasia, and lymphocytic infiltrates in the lamina propria. There is also an increased count of intraepithelial lymphocytes (IELs) that is most obvious in the tips of bowel villi, and this is amongst the earliest histologic findings associated with gluten (23).

The prevalence of CD in first-degree relatives has been directly analyzed using intestinal biopsy in 5 large studies; 3 were carried out in England during the 1970s (24-26), and 2 were conducted in Finland during the 1990s (27,28). The rate of affected relatives oscillated between 34% and 100% (25). Sample size oscillated between 29 and 182 patients. Mean prevalence of CD among first-degree relatives—studied with intestinal biopsy—ranged from 5.5% (25) to 22.5% (26), with a cumulative mean of 16%.

A serologic screening of first-degree relatives has been conducted in various studies, demonstrating a mean prevalence around 12% (29); however, when Marsh I lesions are also included this prevalence goes up to 44.1% (30), which closely resembles those in the present paper.

The prevalence of CD seems higher in relatives with multiple known cases (31), and some authors hardly encounter any difference in CD prevalence between second-degree relatives –19.5% (95% CI, 15.1-23.9)– and first-degree family members –17% (95% CI, 6.4-27.7) (32).

Age has also been seen to influence results, since initially negative individuals were often seen to become positive during follow-up if they had an appropriate genetic predisposition (33).

CD is associated with intestinal lymphoma and other digestive malignancies, particularly small-bowel, pharyngeal, and esophageal adenocarcinomas.

In a series of 175 intestinal malignancies compiled in England CD was associated in 13% of cases, and gluten-related enteropathy had been first diagnosed in 63% of patients (with an 8.2-year interval between both diagnoses) (34).

In our index patient this interval between both diagnoses was similar, if somewhat shorter (7 years).

As with this series, our patient had a proximally located (duodeno-jejunal) small-bowel adenocarcinoma...
associated with intestinal obstruction manifestations and gastrointestinal bleeding. When surgical resection is feasible survival is slightly longer than for intestinal lymphoma.

In a recently published epidemiological study conducted in Sweden in a cohort of 11,000 celiac individuals who were followed for 31 years, the authors found an increased risk for oropharyngeal cancer, with an standardized incidence ratio (SIR) of 2.3 (4.2 for esophageal cancer). They also found a slight increase for primarily proximal colon cancer at 1.5, but not so for descending colon and rectal cancer (35).

Family studies are thus mandatory, including the highest possible number of both second- and first-degree relatives—in special cases luck may have it that all members in a small sample are affected (36); in this way many autoimmune conditions and several malignancies may be prevented; also GFD noticeably improves quality of life for celiac patients, as has been recently demonstrated by Dr. Casellas et al. (37).

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