Current Approaches to Diagnosis and Treatment of Celiac Disease: An Evolving Spectrum

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Celiac disease (CD) is a syndrome characterized by damage of the small intestinal mucosa caused by the gliadin fraction of wheat gluten and similar alcohol-soluble proteins (prolamines) of barley and rye in genetically susceptible subjects. The presence of gluten in these subjects leads to self-perpetuating mucosal damage, whereas elimination of gluten results in full mucosal recovery. The clinical manifestations of CD are protean in nature and vary markedly with the age of the patient, the duration and extent of disease, and the presence of extraintestinal pathologic conditions. In addition to the classical gastrointestinal form, a variety of other clinical manifestations of the disease have been described, including atypical and asymptomatic forms. Therefore, diagnosis of CD is extremely challenging and relies on a sensitive and specific algorithm that allows the identification of different manifestations of the disease. Serologic tests developed in the last decade provide a noninvasive tool to screen both individuals at risk for the disease and the general population. However, the current gold standard for the diagnosis of CD remains histologic confirmation of the intestinal damage in serologically positive individuals. The keystone treatment of CD patients is a lifelong elimination diet in which food products containing gluten are avoided.

Celiac disease (CD) is an autoimmune enteropathy triggered by the ingestion of gluten-containing grains in susceptible individuals. The gliadin fraction of wheat gluten and similar alcohol-soluble proteins in other grains are the environmental factors responsible for the development of the intestinal damage. The disease is associated with HLA alleles DQA1*0501/DQB1*0201, and in the continued presence of gluten the disease is self-perpetuating.[1] The typical intestinal damage characterized by loss of absorptive villi and hyperplasia of the crypts completely resolves upon elimination of gluten-containing grains from the patient's diet.

It is now evident that CD is the result of an inappropriate T cell–mediated immune response against ingested gluten.[2] Under physiologic circumstances, the intestinal epithelium with its intact intercellular tight junctions serves as the main barrier to the passage of macromolecules such as gluten. During this healthy state, quantitatively small but immunologically significant fractions of antigens cross the defense barrier. These antigens are absorbed across the mucosa along 2 functional pathways. The vast majority of absorbed proteins (up to 90%) cross the intestinal barrier through the transcellular pathway, followed by lysosomal degradation, which converts proteins into smaller, nonimmunogenic peptides. The remaining portion of peptides is transported as intact proteins, resulting in antigen-specific immune responses. This latter phenomenon uses the paracellular pathway that involves a subtle but sophisticated regulation of intercellular tight junctions that leads to antigen tolerance. When the integrity of the tight junction system is compromised, such as in CD,[3,4] an immune response to environmental antigens (i.e., gluten) may develop. The up-regulation of zonulin, a recently described intestinal peptide involved in tight junction regulation,[5] seems to be responsible, at least in part, for the increased gut permeability characteristic of the early phase of CD.[6] This zonulin-dependent increased permeability may also be responsible for the increased incidence of autoimmune disorders reported in untreated CD patients.[7]

Another important factor for the intestinal immunologic responsiveness is the major histocompatibility complex (MHC). Human leukocyte antigen (HLA) class I and class II genes are located in the MHC on chromosome 6. These genes code for glycoproteins, which bind peptides, and this HLA–peptide complex is recognized by certain T-cell receptors in the intestinal mucosa.[8,9] Susceptibility to at least 50 diseases, including CD, has been associated with specific HLA class I or class II

Abbreviations used in this paper: AEA, antiendomysium antibody; AGA, antigliadin antibody; CD, celiac disease; ESPGHAN, European Society of Pediatric Gastroenterology, Hepatology, and Nutrition; GFD, gluten-free diet; Ig, immunoglobulin; tTG, tissue transglutaminase.

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Table 1. Possible Clinical Manifestations of CD

Typical symptoms	Atypical symptoms	Associated conditions
Chronic diarrhea	Secondary to malabsorption	Possibly gluten dependent
Failure to thrive	Sideropenic anemia	IDDM
Abdominal distention	Short stature	Autoimmune thyroiditis
	Osteopenia	Autoimmune hepatitis
	Recurrent abortions	Sjögren syndrome
	Hepatic steatosis	Addison disease
	Recurrent abdominal pain	Autoimmune atrophic gastritis
	Gaseousness	Autoimmune emocytopenic diseases
	Independent of malabsorption	Gluten independent
	Dermatitis herpetiformis	Down syndrome
	Dental enamel hypoplasia	Turner syndrome
	Ataxia	Williams syndrome
	Alopecia	Congenital heart defects
	Primary biliary cirrhosis	IgA deficiency
	Isolated hypertransaminasemia	
	Recurrent aphthous stomatitis	
	Myasthenia gravis	
	Recurrent pericarditis	
	Psoriasis	
	Polyneuropathy	
	Epilepsy (with or without intracranial calcifications)	
	Vasculitis	
	Dilatative cardiomyopathy	
	Hypo/hyperthyroidism	

alleles. The primary HLA association in CD is to the HLA-DQA1*0501, DQB1*0201 genes encoding DQ2 molecules.[1] Non-HLA genes together appear to contribute more to genetic susceptibility than do the HLA genes, but the contribution from each single, predisposing non-HLA gene appears to be modest.[10] Dieterich et al.[11] recently demonstrated that one of the targets of the autoimmune response in CD is the tissue transglutaminase (tTG).[11] The deamidating activity of this enzyme seems to generate gliadin peptides that bind to DQ2 to be recognized by disease-specific intestinal T cells.[10]

Clinical Presentations

The clinical manifestations of CD vary markedly with the age of the patient, the duration and extent of disease, and the presence of extraintestinal pathology (Table 1). Depending on the features at the time of presentation, together with the histologic and immunologic abnormalities at the time of diagnosis, CD can be subdivided into the following clinical forms.

Classical (Typical) Form

The onset of symptoms in the classical form generally occurs between 6 and 18 months of age. This form is typically characterized by chronic diarrhea, failure to thrive, anorexia, abdominal distention, and muscle wasting. Growth is usually normal during the first months of life. Symptoms begin within weeks to a few months after the introduction of weaning foods containing prolamines, and soon there is a progressive decrease in weight gain with a decline in the child's percentile for weight and weight for height. On examination, the children are often pale and noticeably thin with a protuberant abdomen, decreased subcutaneous fat, and reduction in muscle mass. The stools are characteristically pale, loose, bulky, and highly offensive because of fat malabsorption. In the very young infant with early onset of symptoms there may be frank watery diarrhea with dehydration and electrolyte imbalance. A small number of these infants also have severe hypoproteinemia and edema and may present in a shocklike state that has been termed "celiac crisis." Laboratory signs of the malabsorption include iron deficiency anemia, hypoalbuminemia, hypocalcemia, and vitamin deficiencies. The pathologic changes are most marked in the duodenum and upper jejunum, but the extent of mucosal damage is highly variable, and in some cases the entire small intestine may be involved. The histologic changes in CD range from minor villous blunting to subtotal or total villous atrophy (see below, Figure 5), decreased villous height-to-crypt depth ratio, crypt hyperplasia with increased mitosis, significantly increased plasma cell and lymphocyte infiltration in the lamina propria, and a pronounced increase in the number of intraepithelial lymphocytes.

Atypical Forms

In recent years there has been a noticeable change in the age of onset of symptoms and the clinical presentation of CD. Mäki et al.[12] first reported an up-shift of age at diagnosis in Finland to 5–6 years, with fewer than 50% of new cases presenting with typical gastrointestinal symptoms. Reports from Scotland,[13] England,[14] Canada,[15] and the United States[16] have also shown that almost 50% of patients with newly diagnosed CD do not present with gastrointestinal symptoms.

Dermatitis herpetiformis. Dermatitis herpetiformis is currently regarded as a variant of CD ("skin CD"). It is a blistering skin disease characterized by pathognomonic granular immunoglobulin (Ig) A deposits in uninvolved skin.[17] The most typical sites of the rash are the elbows, knees, and buttocks. Intestinal symptoms are not common, but a varying degree of enteropathy, ranging from the infiltrative-type lesion to flat mucosa, can be found on small intestinal biopsy in almost 100% of cases. Both the enteropathy and the rash slowly clear with a gluten-free diet (GFD) and relapse when patients return to a regular diet.[18]

Iron-deficiency anemia. Iron deficiency with or without anemia, typically refractory to oral iron supplementation, can be the only presenting sign of CD.[19]

Short stature. Short stature is well described as the only symptom of CD in some older children and adolescents, and it is believed that as many as 9%–10% of those with "idiopathic" short stature have CD.[20–22] In these patients, both the bone age and growth velocity are significantly impaired.[20,22,23] Some patients have also demonstrated impaired growth hormone production after provocative stimulation testing.[23] This value returns to normal after introduction of a GFD.[24]

Dental enamel hypoplasia. Dental enamel hypoplasia has been found in up to 30% of untreated patients with CD.[25,26]

Arthritis and arthralgia. CD has been described in 1.5%–7.5% of patients with rheumatoid arthritis.[27–29] These symptoms were reported by Mäki et al.[30] as the only presentation of CD in 7 adolescent patients. In each case, the symptoms resolved on introduction of a GFD and all other anti-inflammatory medications could be discontinued.

Chronic hepatitis and hypertransaminasemia. Idiopathic chronic hepatitis as the initial presentation of CD has been reported occasionally.[31,32] Vajro et al.[33] describe 3 children with cryptogenetic chronic hepatitis secondary to CD. In all cases, GFD induced complete remission with normalization of the biochemical and histologic changes of hepatitis. Resolution of the biochemical abnormalities associated with hepatic damage has been reported in a high percentage of pediatric patients with CD who adhered to a strict GFD.[34]

Osteoporosis. Patients with CD are at high risk for developing low bone mineral density and bone turnover impairment. Persistent villous atrophy is associated with low bone mineral density. In adult patients responsive to diet, the bone density seems comparable to that of healthy individuals.[35] Children who followed a GFD for at least 5 years had normal bone mineralization and bone turnover.[36] Of 86 consecutive patients with newly diagnosed, biopsy-confirmed CD, 40% had osteopenia and 26% osteoporosis.[36] No differences between male and female patients or between fertile and postmenopausal women were observed. Even in postmenopausal women, GFD led to significant improvement in bone mineral density.[37] In these cases, supplement treatment with vitamin D and Ca^{2+} is indicated.

Neurologic problems. Gluten sensitivity is common in patients with neurological diseases of unknown cause and may have etiologic significance.[38] Pellecchia et al.[39] recently reported that 3 of 24 patients with idiopathic cerebellar ataxia had CD.

Other extragastrointestinal symptoms. A delay in onset of puberty secondary to CD has been described in a number of adolescent patients.[12,22,40,41] Recurrent abortions[42,43] and reduced fertility[40,42] caused by CD have also been reported in this age group. Recently, Ciacci et al.[44] have reported that the relative risk of abortion in women affected by CD is 8.9 times higher than in healthy subjects, and a GFD reduced the relative risk of abortion.[44]

Asymptomatic (Silent) Form

This form is characterized by the presence of histologic changes, probably limited to the proximal intestine, that occur in individuals who are apparently asymptomatic.[45–47] Most cases in this category have been identified through screening programs involving apparently healthy subjects. However, a more careful clinical anamnesis typically reveals that many of these "silent" cases are indeed affected by low-intensity illness often associated with decreased psychophysical well-being. Common findings include (1) iron deficiency with or without anemia; (2) behavioral disturbances, such as tendency to depression, irritability, or impaired school performance in children; (3) impaired physical fitness, "feeling always tired," and easy fatigue during exercise; and (4) reduced bone mineral density.[48,49] A 24-month follow-up study showed that adolescents with screening-detected CD who were apparently symptomless at diagnosis often reported improved physical and psychologic

conditions once they began following a GFD.[50] The most common changes included increased weight and height velocity, increased appetite, mood amelioration, and improved physical and school performance.[50] Finally, current evidence suggests that subjects with "silent" CD are at risk to develop the same long-term complications experienced by individuals with typical symptoms.

Associated Diseases

A number of medical conditions are significantly associated with CD (Table 1). For some of these conditions, sensitivity to gliadin has been conclusively proven or may be implicated (Table 1).

Complications Associated With Unrecognized CD

Malignancies. The persistence of mucosal injury with or without typical symptoms can lead to serious complications, and gastrointestinal malignancies (particularly lymphoma) have been reported in 10%–15% of adult patients with known CD who do not strictly comply with a GFD.[51] However, the increased risk for malignancy in the gastrointestinal tract in patients with CD has been questioned recently; therefore, the precise magnitude of this complication remains uncertain (see diagnosis section below). Nevertheless, it has been reported that the mortality rate in CD patients is almost double (1.9×) the rate calculated for the general population, mainly because of the occurrence of neoplasms.[52] Data from Logan et al.[52] have shown that when appropriate treatment for CD was instituted in childhood and strictly followed, the mortality rate of these subjects was no different from that expected in the general population, and no deaths from intestinal lymphoma were recorded.

Autoimmune diseases. CD seems to meet the criteria of a true autoimmune disease for which the genetic predisposition (HLA), exogenous trigger (gluten), and autoantigen (tTG) are known. It seems that tTG is only one of the autoantigens involved in gluten-dependent autoimmune reactions. Other autoantigens that are normally "cryptic" can be unmasked and cause a self-aggressive immunologic response following the gliadin-initiated inflammatory process. In fact, persistent stimulation by some proinflammatory cytokines such as interferon γ and tumor necrosis factor α can cause further processing of autoantigens and their presentation to T lymphocytes by macrophage-type immunocompetent cells (the so-called antigen-presenting cells). The phenomenon of antigen spreading has been described in well-defined natural models such as insulin-dependent diabetes mellitus, whose clinical manifestations appear after the patient has produced an autoimmune response to various autoantigens (i.e., anti-insulin, anti–β cell), and might also be present in CD. This could explain the high incidence of autoimmune diseases (Table 1) and the presence of a large number of organ-specific autoantibodies in a certain number of celiac subjects on a gluten-containing diet.

Based on this evidence, it is tempting to hypothesize that the range of gluten-dependent autoimmune disorders present in genetically predisposed individuals goes well beyond the classic enteropathy of CD (Table 1). Furthermore, recent data suggest that the prevalence of autoimmune diseases among patients with CD is proportional to the time of exposure to gluten.[7]

The Epidemiology of CD

Epidemiology of CD in Europe

In the past 3 decades, a substantial number of epidemiologic studies have been conducted in Europe to establish the frequency of CD, and interesting controversies have arisen. Earlier investigations measured the incidence of CD, namely the number of "new" diagnoses in the study population during a certain period. One of the oldest epidemiologic studies on CD conducted in 1950 established that the cumulative incidence of the disease in England and Wales was 1/8000, whereas an incidence of 1/4000 was detected in Scotland.[53] The diagnosis at that time was entirely based on the detection of typical symptoms and confirmed by complicated and sometimes nonspecific tests. The awareness of the disease greatly increased in the 1960s when more specific tests for malabsorption and the pediatric peroral biopsy technique became available.[54] Consequently, an elevated incidence of the disease (which in the middle 1970s reached peaks of 1/450–500) was reported in studies from Ireland,[55] Scotland,[56] and Switzerland.[57] This increased incidence of CD prompted changes in the dietary habit, based on the hypothesis that delayed exposure to gluten could prevent the onset of the disease. For the first time in 25 years, a decrease in the incidence of CD was reported in the United Kingdom and Ireland[58–60] after a late introduction of gluten in infant diet. Unfortunately, this decrease was deceptive because subsequent screening studies showed that the reduction in typical cases in infants was counterbalanced by the increase of atypical forms of CD with the onset of the symptoms occurring in older children or in adults.[61] Because of the development of sensitive serologic tests, it has recently become possible to evaluate the prevalence of CD (number of affected persons, including subclinical cases, in a defined popu-

Table 2. Prevalence of CD Based on Clinical Diagnosis or Screening Data

Geographic area	Prevalence on clinical diagnosis[a]	Prevalence on screening data
Brazil	?	1:400
Denmark	1:10,000	1:500
Finland	1:1000	1:130
Germany	1:2300	1:500
Italy	1:1000	1:184
Netherlands	1:4500	1:198
Norway	1:675	1:250
Sahara	?	1:70
Slovenia	?	1:550
Sweden	1:330	1:190
United Kingdom	1:300	1:112
United States	1:10,000	1:111
Worldwide (average)	1:3345	1:266

[a]Classical gastrointestinal symptoms.
Data from references 81–85, 125–131.

lation at a certain point). Screening studies show a high prevalence of CD among both healthy children[62–64] and adults.[65] The prevalence of CD throughout the old continent seems to be more homogeneous than previously thought (Table 2). Furthermore, these screenings showed that CD is one of the most frequent genetically based diseases,[62,66] occurring in 1 of 130–300 in the European population[67,68] (Table 2). In a serologic screening study involving more than 17,000 Italian schoolchildren, the prevalence of CD was 1 in 184,[48] and the ratio of known to undiagnosed CD cases was 1 to 7. The European experience taught that, despite common genetic and environmental factors, the clinical presentation of CD in neighboring countries may greatly diverge. A typical example of this phenomenon is the Danish epidemiologic case. Until a few years ago, CD was regarded as rare in Denmark, with an estimated incidence based on clinical evidence (i.e., presence of classical symptoms) of 1/10,000[69] (Table 2). At the same time, the incidence of the disease in neighboring countries (including Sweden and Finland) that share similar genetic backgrounds increased after a decrease in breast feeding practice and increased consumption of gluten during infancy.[70,71] Subsequent serologic screening studies suggested that CD is as frequent in Denmark as in Sweden, with a reported prevalence of 1/500[72] (Table 2). These results suggest that in Denmark most cases of CD were previously undiagnosed, presumably because of lack of typical gastrointestinal symptoms. Factors such as type of cow's milk formulas, breast feeding, age at gluten introduction, quantity of gluten and quality of cereals, and quantity of wheat gluten may all influence the clinical presentation of the disease.[71]

Epidemiology of CD in the United States

In the American scientific community it is generally believed that CD is a rare disorder in the United States, which is reflected by the limited number of scientific papers published from the new continent in the 30-year period from 1965 to 1995.[73] Only 2 epidemiologic studies of CD were published during this period, both between 1993 and 1994. The first study was conducted by Rossi et al.[74] in 1993 on a pediatric population from the western New York area with symptoms possibly related to CD, such as chronic diarrhea, failure to thrive, short stature, and diabetes.[74] Although the prevalence of CD among patients with symptoms possibly associated to the disease was lower than reported in Europe, the concurrence of CD and insulin-dependent diabetes mellitus was comparable to that previously reported from the old continent. These data suggest that other atypical presentations of CD and eventually late onset of the disease after an asymptomatic phase during childhood may account for the low occurrence of CD reported in this study. The second American epidemiologic study published in 1994 was based on a retrospective evaluation (1960–1990) of the incidence of CD among the population of Olmsted County, Minnesota, using the medical record of the Rochester Epidemiological Project.[75] Case definition was limited to those individuals presenting typical gastrointestinal symptoms (i.e., chronic diarrhea and weight loss) or dermatitis herpetiformis whose intestinal biopsies showed flat mucosa.[75] Using these restrictive parameters, the authors identified only 3 cases among the pediatric population (calculated incidence rate, 0.4 per 100,000 person-years), whereas the overall age- and gender-adjusted incidence was 1.2 per 100,000 person-years. Based on these results, the authors concluded that CD is relatively rare in the United States (prevalence ~1:10,000). Unfortunately, both studies failed to consider the protean clinical manifestations of CD. By focusing on specific symptoms, the authors may have missed what is currently defined as the submerged part of the so-called celiac iceberg (Figure 1). Recently, a series of epidemiologic studies conducted using more appropriate experimental designs and powerful screening tools showed that CD is as frequent in the United States as in Europe in both risk groups[76–78] and the general population[79,80] (Table 2). Our center for celiac research is currently conducting a large, multicenter study on the prevalence of CD in both risk groups (i.e., subjects with either symptoms or complications associated with CD, first- and second-degree relatives of patients with biopsy-proven CD, etc.) and the general population. The results generated on a large number of

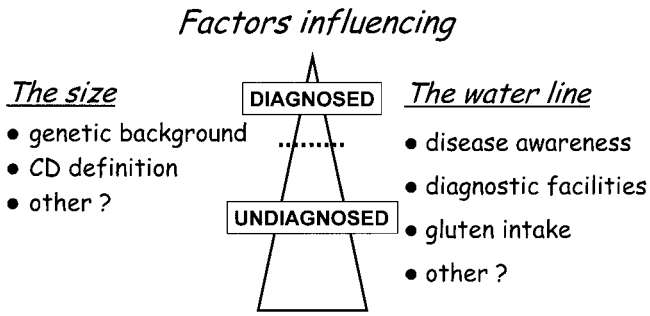

Figure 1. The CD iceberg model.

individuals screened so far suggest that the prevalence of CD in the United States is similar to that reported in Europe if not even higher, both among risk groups and in the general population[81] (Table 2).

Epidemiology of CD in the Rest of the World

Because CD is the result of the interaction between genetic (both HLA and non–HLA-associated genes) and environmental factors (gluten-containing grains), it would be reasonable to evaluate the world distribution of these 2 components to identify areas "at risk" for CD. The coincidence of the CD HLA aplotypes (Figure 2*A*) and the level of wheat consumption (Figure 2*B*) clearly confirm Europe as a region at risk for CD. However, the coexistence of the 2 key components involved in CD pathogenesis (Figure 2*A* and *B*) is also notable in regions where CD has been historically considered rare. This apparent paradox can be explained by the limited number of scientific studies performed in some of those countries in which CD is perceived as a rare disorder (Figure 2*C*). Recent epidemiologic studies conducted in areas at risk (Figure 2*A* and *B*), such as South America,[82] North Africa,[83] and Asia,[84,85] suggest that CD was indeed underdiagnosed.

Combined together, these studies suggest that CD is still underestimated in areas where large epidemiologic studies are lacking. The European experience taught us that despite common genetic and environmental factors, the clinical presentation of CD in neighboring countries may greatly diverge and could explain the different disease prevalence previously reported. A comparison between the estimated prevalence (based on the occurrence of typical symptoms) and the serologic screening data (where available) shows that CD is a common disease but its gastrointestinal presentation is relatively rare, particularly in countries in which CD was considered a negligible pathology (Table 2). Worldwide, CD "out of the intestine" is 15 times more frequent than CD "in the intestine" (Table 2), making the diagnosis extremely challenging.

The Iceberg Model

The epidemiological changes of CD are efficiently conceptualized by the iceberg model, originally introduced by Richard Logan in 1991.[86] The prevalence of CD can be conceived as the overall size of the iceberg, which is primarily influenced by the frequency of the predisposing genotypes in the population. Indeed, CD seems to be more common wherever the frequency of the HLA-DR3 (and DQ2) is high, such as in Europe, the United States, and North Africa. The dimension of this iceberg also depends, to a lesser extent, on disease definition, i.e., whether subjects with so-called latent or potential CD[47] or those with gluten sensitivity and mild enteropathy[87] are "counted" as affected individuals. In countries where a substantial part of the population is of European origin, the prevalence of CD is likely to be more stable than previously thought, roughly in the range of 0.5%–1% of the general population. A sizable number of these cases are properly diagnosed because of suggestive complaints (e.g., chronic diarrhea, unexplained iron deficiency) or other reasons (e.g., family history of CD). These cases make up the visible part of the celiac iceberg, in quantitative terms expressed by the incidence of the disease. However, as previously reported, screening studies show that in Western countries, for each diagnosed case of CD, an average of 5–10 cases remain undiagnosed (the submerged part of the iceberg). The "water line," namely the ratio of diagnosed to undiagnosed cases, depends on several factors: (1) awareness of CD: "think of CD and you will find it" is an aphorism worth remembering[88]; differing awareness, and consequently variable thresholds for serologic CD testing, is likely to explain a substantial part of the wide differences in incidence between countries; (2) availability of diagnostic facilities: lack of both laboratory equipment and personnel trained in CD diagnosis is a major problem in large areas of the world, e.g., North Africa, the Middle East, and India, where the frequency of CD is currently underestimated; (3) variations in clinical intensity: at both individual and population levels, the higher the amount of ingested gluten, the higher the intensity of the clinical picture, thereby increasing the chances that CD can be diagnosed on clinical grounds. This has been clearly shown by the "epidemic" of CD observed in Sweden during the 1980s and early 1990s, in relationship with the gluten load that infants received with follow-up formulas.[89] Because of the variable relevance of these factors, the water line is much more unstable than the overall size of the iceberg, thereby explaining the reported wide fluctuations in space and time of CD incidence. What remains to be evaluated is the effect of

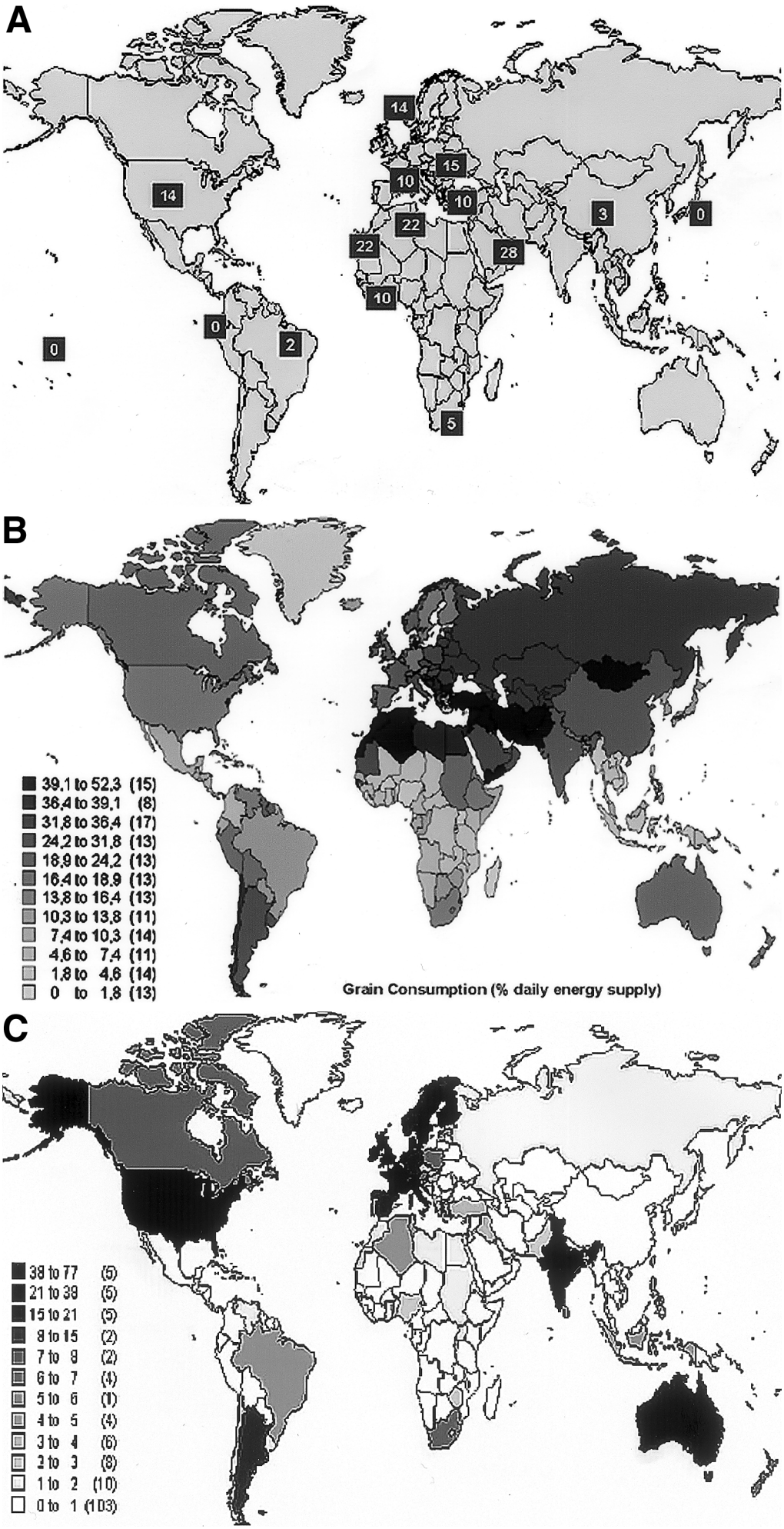

Figure 2. (*A*) CD-associated HLA-DR3. Percentage of genic frequency of HLA-DR3 in the world. (Data provided by Dr. Francesco Cucca, Department of Pediatrics, University of Cagliari, Cagliari, Italy.) (*B*) World distribution of grain consumption. The intensity of color is directly related to the amount of wheat products consumed (expressed as percent of daily energy supply). Numbers in parentheses represent the number of countries that have the wheat consumption shown on the left. (Data from The Sixth World Food Survey; Rome, Italy: Food and Agriculture Organization of the United Nations, 1996.) (*C*) Scientific production on CD worldwide during the period from 1966 to the present. The intensity of color is directly related to the number of articles found in a MEDLINE search for CD and the name of the country. Numbers in parentheses represent the number of countries that have published within the range of manuscripts shown on the left.

other factors (e.g., intestinal infections and nutrient intakes) on the clinical presentation and, even more intriguingly, whether environmental variables can influence the prevalence of CD, therefore assessing the fascinating possibility of primary prevention of this disorder.

How to Diagnose CD?

The diagnosis of CD is based on 3 key parameters: (1) case identification, (2) screening tests, and (3) definitive tests. These parameters have substantially changed during the past 50 years, thanks to better understanding of the clinical presentation of the disease and the advent of more sensitive and specific diagnostic tools and confirmative tests.

The Past

Until a few decades ago, there was the general perception that the clinical presentation of the disease was quite uniform. Case identification was based entirely on the search for symptoms such as chronic diarrhea, abdominal distention, and weight loss (or poor weight gain) occurring in young children a few months after the introduction of solid food to their diet. To confirm clinically suspected CD, unspecific screening tests aimed at establishing the digestive/absorptive functions of the proximal small intestine (i.e., glucose tolerance test, D-xylose test, fecal fat) were used. Given the lifelong nature of the disease, in 1970 the European Society of Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) dictated specific guidelines by identifying 3 CD diagnostic phases (Figure 3).[90] To meet the criteria of the first phase, the presence of gastrointestinal symptoms compatible with CD, positive results of pathologic screening tests, and confirmation of the diagnosis by intestinal biopsy showing histologic evidence of flat mucosa were required (Figure 3). Upon establishment on a GFD, the clinical symptoms had to resolve, results of screening tests had to return to within normal limits, and a second intestinal biopsy showing complete healing of the histologic damage was recommended (phase 2) (Figure 3). Phase 3 was then started by a gluten challenge with subsequent return of symptoms, pathologic screening test results, and intestinal damage (Figure 3). The diagnosis was confirmed only if all the criteria listed in the 3 phases were completely satisfied.

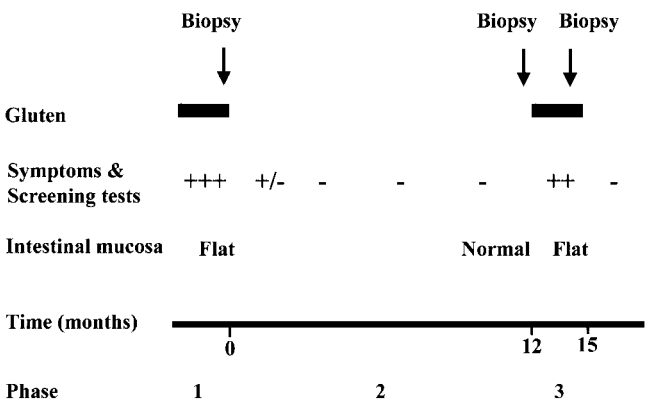

Figure 3. CD diagnostic protocol proposed by the ESPGHAN in 1970.

The Present

Development of serologic tests. In the past 10–15 years we have learned that the clinical expression of CD is more heterogeneous than previously thought.[86] Beside the classical gastrointestinal form, a series of other clinical manifestations of the disease have been described thanks to the advent of innovative serologic screening tests, such as assays for antigliadin antibody (AGA) and antiendomysium antibody (AEA). The combined use of serum AGA IgG (good sensitivity) and IgA (good specificity) resulted in a reliable screening test for diagnosis of CD.[91] Based on the use of this new tool, we have learned that the clinical presentation of CD is more protean than previously thought, including previously unrecognized atypical and asymptomatic forms (see above). Moreover, these studies show that CD is not limited to the pediatric population; the onset of disease may occur during adulthood, after years of silent disease.

Because it has been demonstrated recently that tTG is the target of a specific autoimmune response (see below),[11] this enzyme has also been used to develop innovative diagnostic tools. The routine use of the AEA assay is limited by elevated costs, time-consuming protocols unsuitable for testing large numbers of samples, poor sensitivity in young children (<2 years of age) and in IgA-deficient individuals (the AEA assays routinely performed are of the IgA class), and use of the esophagus of an endangered species (such as the monkey) as the substrate for the immunofluorescent analysis. Even if this last issue has been resolved by using the human umbilical cord as a valid alternative to the monkey esophagus,[80] it has been reported that the subjective interpretation of the AEA assay may lead to unacceptable variability among laboratories that perform this test.[92] Therefore, major effort has been concentrated on developing a tTG-based ELISA, using either the commercially available guinea pig tTG[93,94] or human recombinant tTG.[95,96]

The currently available serologic tests for the diagnosis of CD remain within the province of the specialized diagnostic laboratory. Given the projected high prevalence of the disease and its protean nature, a simple

diagnostic test that could be used at the general practitioner's site would represent a great advance. The recent report of a human tTG dot blot test based on the detection of anti-tTG antibodies in serum or in 1 drop of whole blood[97] opened new horizons for the diagnosis of CD. These preliminary results show that the test seems to be extremely sensitive (100%) and reasonably specific (96%). If these data are confirmed, this test holds great potential because it is quick (30 minutes) and inexpensive, requires minimal handling, and in view of its high sensitivity and specificity could easily be introduced into the general practitioner's armory for ambulatory screening of CD.

Current guidelines for serologic diagnosis and follow-up of CD. Because the guinea pig–based tTG ELISA has been only recently commercialized and the human-based tTG ELISA is still experimental, serologic diagnosis of CD still relies on the combined use of AGA and AEA assays. Interpretation of these assays should take into account the fact that AEA can have false-negative results in both IgA-deficient subjects and children younger than 2 years of age, whereas AGA (particularly the IgG subclass) can yield false-positive results in gastrointestinal conditions other than CD, including cow's milk protein intolerance and parasite infections. Once a definitive diagnosis is established (see below), use of these serologic tests is recommended to verify compliance with the GFD, which should be evaluated on a yearly basis or every time patients experience symptoms possibly related to gluten exposure. If the preliminary data so far reported on the sensitivity and specificity of the tTG ELISA (Table 3) are confirmed on a large scale, it is likely that this test will make the AGA and possibly the AEA assays obsolete.

Algorithm for the definitive diagnosis of CD. Given the high sensitivity and specificity reported for some of the screening tools currently available (Table 3), the ESPGHAN has recently proposed a revised CD diagnostic protocol[98] (Figure 4). Based on these revised criteria, if the symptoms (either typical or atypical) and screening results are suggestive, a single intestinal biopsy followed by a favorable response to the GFD is sufficient to definitely confirm the diagnosis (Figure 4). However, total villous atrophy, once considered the only histologic finding compatible with a diagnosis of CD, is now considered only the extreme of a continuous spectrum of tissue damage that can be detected during the acute phase of the disease (Figure 5). Furthermore, the possible patchy characteristics of intestinal damage[99] and the importance of correct orientation of the biopsy for appropriate evaluation of the intestinal damage both add further challenge to a conclusive histologic diagnosis of CD.

Table 3. Sensitivity, Specificity, and Positive and Negative Predictive Values of Serologic Screening Tests Reported in the Literature for the Diagnosis of CD

Test	Sensitivity	Specificity	PPV	NPD
AGA IgG	57–100	42–98	20–95	41–88
AGA IgA	53–100	65–100	28–100	65–100
AEA IgA[a]	75–98	96–100	98–100	80–95
Guinea pig tTG[b]	90.2	95		
Human tTG[b]	98.5	98		

PPV, positive predictive value; NPD, negative predictive value.
[a]Patients older than 2 years.
[b]IgG + IgA antibodies.
Data from references 132–138.

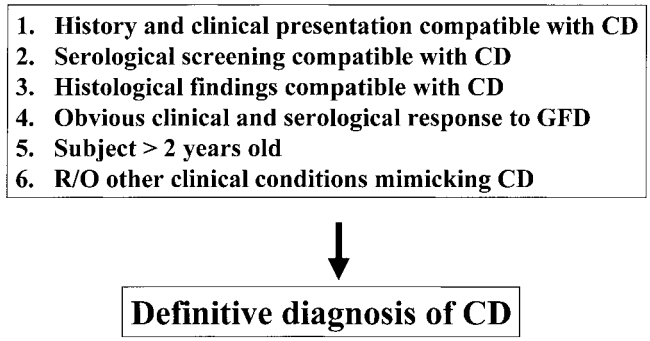

Figure 4. Revised criteria for the diagnosis of CD proposed by the ESPGHAN.

Who Should Be Tested?

At-risk groups. Serologic testing is indicated for subjects with symptoms suggestive of CD, as well as for those with CD-associated diseases (Table 1). However, small intestinal biopsies should always be performed if the clinical suspicion is strong, regardless of the serology results. Some at-risk groups showing a particularly high prevalence of associated CD (Figure 6) deserve a special mention: (1) first- and second-degree relatives of patients with CD: younger siblings can be checked at age 2 years or earlier if CD is clinically suspected; (2) patients and relatives of patients with type I diabetes[100] and patients with immune thyroid or liver disorders; (3) patients with Sjögren syndrome and other connective tissue diseases: in a recent Finnish series, 5 (15%) of 34 patients with Sjögren syndrome were found to have CD,[101] although ongoing inflammation was often present in the small intestinal mucosa of patients without CD[101]; (4) subjects with either Down or Turner syndrome; and (5) subjects with selective IgA deficiency, who show a 10-fold increased risk of associated CD.[102] In these cases, the screening test should be an IgG class antibody, e.g., AGA IgG or anti-tTG IgG.[86]

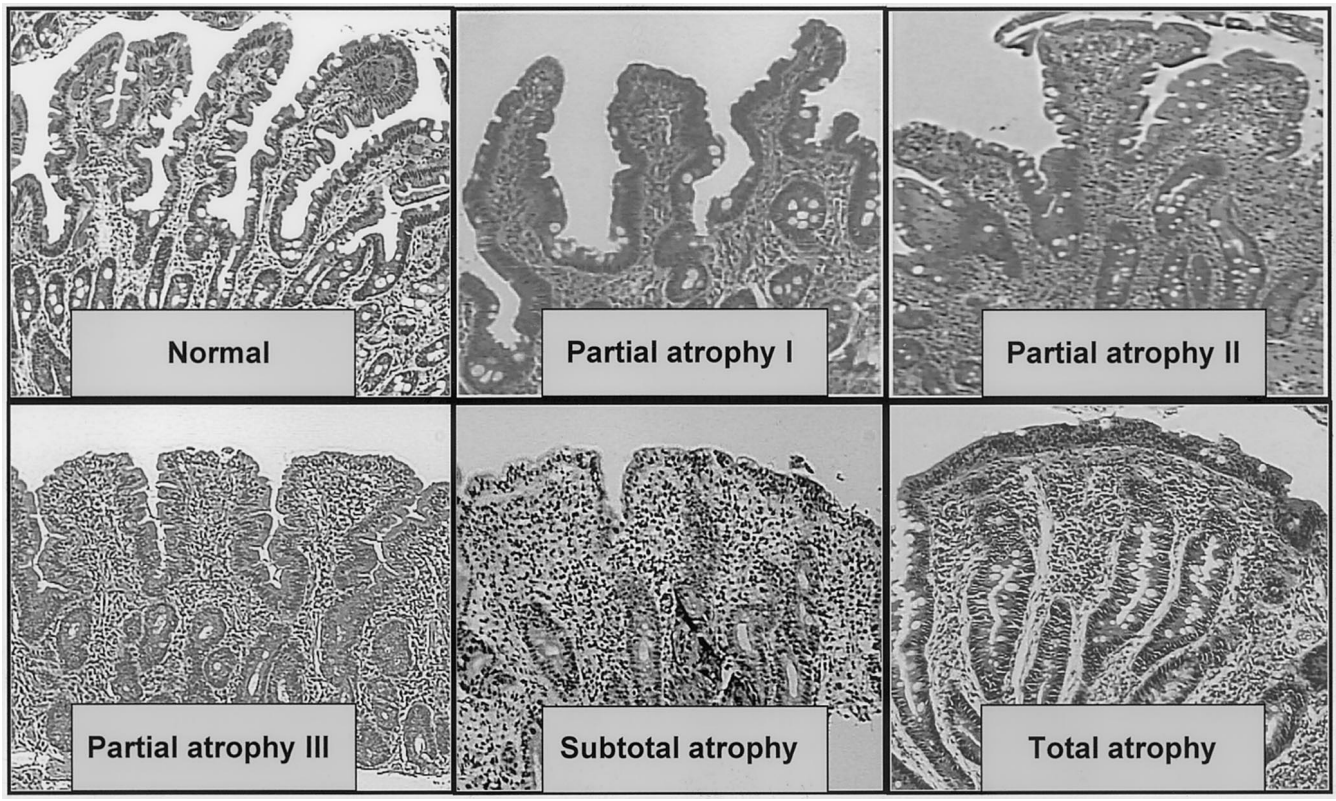

Figure 5. Histologic grades of intestinal mucosa damage in patients with CD (courtesy of Dr. Karoly Horvath).

A single negative result of the serologic markers cannot always rule out the possibility of CD on a lifelong basis. This has been elegantly shown by a recent follow-up study of 275 patients with type I diabetes, in which only 2 of 9 patients found to have CD during a 6-year period had an AEA-positive test result at the time of diabetes onset.[103]

Finally, we suggest that serologic testing for CD should be performed routinely in people joining blood donor groups. Because the celiac enteropathy often impairs iron absorption, CD should be identified as soon as possible in these subjects to avoid the onset of a sideropenic anemia secondary to the combination of periodic blood drawings and the malabsorption condition typical of the disease.

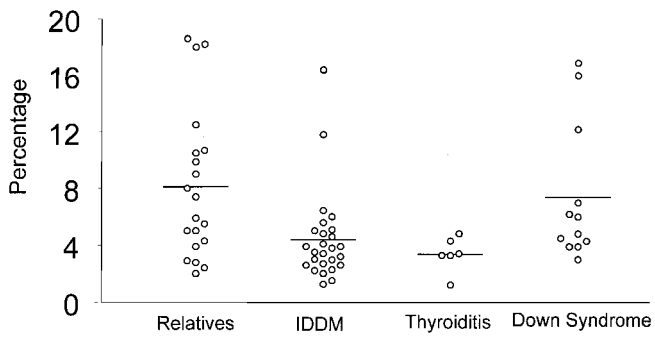

Figure 6. Prevalence of CD in some at-risk groups. *Dots* represent the prevalence found in different studies, and *lines* show the mean values.

Case finding or mass screening? How to deal with the submerged part of the celiac iceberg is currently a matter of debate in the scientific community. An increasing number of experts is in favor of early, mass screening of CD because this condition apparently fulfills the requirements for a worthwhile screening program: (1) it is a common disorder causing significant morbidity in the general population; (2) early detection is often difficult on a clinical basis; (3) if not recognized, the disease can manifest itself with severe complications that are difficult to manage (e.g., infertility, osteoporosis, lymphoma); (4) there is an effective treatment, the GFD; and (5) sensitive and simple screening tests are available, e.g., the anti-tTG test.

However, several issues need further clarification to correctly establish the cost/benefit ratio for CD screening. Although it is well established that patients with untreated CD may develop complications, the natural history of undiagnosed CD is currently unclear. Available studies have necessarily been limited to patients with clinically diagnosed CD (i.e., the tip of the iceberg), eventually leading to biased estimate of the risks. For

example, the relative risk of developing a lymphoma complication was reported to be as high as 30–100,[53,104] whereas an ongoing case-control multicenter Italian study investigating the prevalence of CD in patients with lymphoma seems to indicate only a slight increase in the risk of this malignancy (odds ratio ~2) in comparison with the general population.[105] Despite the high sensitivity of the serologic CD markers, the positive predictive value of these investigations decreases when they are used in the general population rather than in at-risk groups.[106] The appropriate age to screen for CD also remains to be established, as well as whether periodic repetition of the screening would be required to rule out a "late onset" gluten sensitization.[107] Because of the ethical implications of mass screening, the difficulties of treating patients with apparently silent CD should not be overlooked. A recent 5-year follow-up study revealed a 30% decrease in adherence to the GFD in patients with screening-detected CD compared with age-matched, hospital-detected CD cases.[108] Wherever products containing wheat flour represent the staple food, treatment with a GFD is likely to interfere with quality of life, especially in adults, and it has been shown that adults with CD undergoing long-term treatment fail to attain the same degree of subjective health as the general population.[109]

Finally, mass screening for CD will depend on the results of comprehensive, well-performed cost-effectiveness analyses. Currently, the "best buy" approach to the submerged portion of the iceberg of undiagnosed CD seems to be a systemic process of case finding, as suggested by a recent study developed in a primary care setting in central England.[110] By simply investigating at-risk subjects, e.g., those with anemia, fatigue, thyroid disease, diabetes, or a family history of CD, Hin et al. observed a 4-fold increase in the number of CD diagnoses during a 1-year period.[110] Increased awareness of the extraintestinal manifestations of CD, coupled with a low threshold for serologic testing, uncovers a large portion of the submerged iceberg.[110]

Why Early Diagnosis Is Important

Our better understanding on the pathogenesis of CD[2] and the observation that CD patients' risk of developing autoimmune diseases[11] and intestinal lymphomas[51,52] is proportional to the time of exposure to gluten suggest that prompt diagnosis is crucial to minimize if not prevent serious complications. Based on epidemiologic data, it might be hypothesized that if CD develops early with typical gastrointestinal symptoms, prompt diagnosis and thus timely prescription of a GFD are more likely. If, on the other hand, symptoms are atypical or completely absent, diagnosis of CD becomes more difficult and the diet treatment is significantly delayed. In these subjects, exposure to gluten will continue for a prolonged period, with a subsequent increase in the risk of complications.

The Treatment

Total lifelong avoidance of gluten ingestion remains the cornerstone treatment for the disease. The diet requires ongoing education of patients and their families by both doctors and dieticians. Regional CD support groups are instrumental sources of information and support. One of the major controversies in the treatment of CD relates to the amount of gluten allowed in the diet of CD patients. The National Food Authority has recently redefined the term "gluten-free." Previously, <0.02% gluten was considered gluten-free, but gluten-free now means no gluten, and <0.02% is currently labeled "low gluten." However, the stringency of gluten restriction (zero tolerance versus low gluten ingestion) is an issue that is far from being resolved because opinions differ among scientists and CD support groups worldwide. These controversies are attributable to a lack of solid scientific evidence for a threshold of gluten consumption below which no harm occurs. The gliadin fraction of wheat gluten and similar alcohol-soluble proteins (prolamins) in other grains are the environmental factors responsible for the development of intestinal damage. Prolamins are found in a variety of widely used grains (Table 4). Therefore, products labeled "wheat-free" are not necessarily gluten-free. They may contain gluten as well as other grains that are not allowed. Wheat, rye, and barley are the predominant grains containing toxic peptides. Both in vivo challenges and in vitro immunologic studies support the possibility that oats (once considered toxic for CD patients) can be ingested safely.[111] However, because of uncontrolled harvesting and milling procedures, cross-contamination of oats with gluten is a concern. Triticale (a combination of wheat and rye), kamut, and spelt[112] (sometimes called farro) are also toxic. Other forms of wheat are semolina (durum wheat), farina, einkorn, bulgur, couscous, and any form that includes wheat in the name, such as wheat germ, wheat bran, whole wheat, and cracked wheat. Foods made from rye and barley are toxic. Malt is also toxic because it is a partial hydrolysate of barley prolamins. It may contain 100–200 mg of barley prolamins /100 g of malt.[113] In general, an ingredient with malt in its name (barley malt, malt syrup, malt extract, malt flavorings) is made from barley.

Table 4. General Guidelines for the CD Diet

Not allowed		Allowed
Wheats (*Triticum* family)		Rice, wild rice
All forms, including	Einkorn wheat (*Triticum monococcum*)	Corn (maize)
Wheat flour		
Wheat germ		
Wheat bran		
Cracked wheat		
	Emmer wheat (*Triticum dicoccon*)	Sorghum
	Couscous (endosperm of durum wheat)	Millet
	Kamut (*Triticum polonicum*)	Buckwheat (kasha)
	Spelt (farro, drinkle)	Beans, peas, and bean flours
	Semolina (durum wheat)	Quinoa
Rye (*Secale cereale*)		Potato
Triticale (wheat-rye hybrid)		Soybean
Barley (*Hordeum vulgare*) and malt		Tapioca
		Amaranth
		Teff
		Nuts
		Fruits
		Milk (cheeses[a])
		Plain meat
		Fish
		Egg
		Oat (*Avena sativa*)[b]

[a]The coat of some cheeses may contain gluten.
[b]Awaiting definitive scientific confirmation and regulation to avoid cross-contamination.

Gluten in Medications

Medications and vitamin and mineral supplements may also contain gluten as an inactive ingredient. The inactive ingredients of these products can be changed by the manufacturers without warning because there are no regulations on the formulation of inactive drug components. Nebulous (questionable) ingredients, such as vegetable gum and modified food starch, can contain gluten. All medications should be checked for nebulous ingredients, especially if they must be taken for a long period. It is imperative to know the lot number of nonprescription medications when contacting the manufacturer for clarification of the inactive ingredients.

Prescription medications purchased through a pharmacy come with an ingredient list on the package insert. However, different batches of medications may contain different ingredients.

The limited expertise of health care professionals regarding celiac diet and the absence of federal regulations for accurate food and drug labeling both represent significant challenges for patients with newly diagnosed CD. Despite the efforts of celiac support groups, there are still no laws regulating gluten-free labeling in the United States. The American Dietetic Association's National Center for Nutrition and Dietetics Consumer Nutrition Hotline at 1-800-366-1655 is a valuable source of updated information on the treatment of CD. One of the functions of the Consumer Nutrition Hotline is to refer consumers and health care professionals to registered dietitians who have expertise in special diseases. The Consumer Nutrition Hotline can also provide phone numbers and addresses of companies within the food industry to help clarify the ingredients of a given food product and how it has been processed.

Problems in Practical Dietary Management

Possible gluten contamination of products that are presumed to be gluten-free is a recurrent problem. This cross-contamination can happen in farms where the grains are grown and harvested, in mills where grains are processed into flours, or on food processing lines where one line produces a food that includes gluten and the line next to it produces a gluten-free product. Contamination might also occur in stores where grains are available from open bins, in restaurants, at salad bars, or any place where a variety of different meals are produced or different ingredients come together.[114]

Refractory Sprue

In a minority of adult patients, CD does not respond to treatment with a gluten-free diet. The most likely cause of nonresponsiveness is continued gluten ingestion, which can be voluntary or inadvertent. Other causes of nonresponsiveness that must be considered include other food intolerance diseases (e.g., milk, soya), pancreatic insufficiency, enteropathy-associated T-cell

Table 5. Research Priorities Identified at the 9th International Symposium on CD

Area of research
Searching for the CD genes
Developing a vaccine against CD
Who, when, and how to screen for CD
Engineering gluten-free grains
Gaining more insight on CD pathogenesis
Developing noninvasive, fast, and reliable tests for the diagnosis and follow-up of CD
Web information
http://www.celiaccenter.org
http://www.nowheat.com/grfx/nowheat/index.htm
http://www.niddk.nih.gov/health/digest/pubs/celiac/index.htm
http://www.fastlane.net/homepages/thodge/archive.shtml

lymphoma, refractory sprue, and ulcerative jejunitis. Patients with CD in whom the lack of compliance to a GFD has been ruled out belong to the refractory sprue category. An aberrant clonal intraepithelial T-cell population can be found in up to 75% of patients with refractory sprue, a condition that is currently classified as cryptic enteropathy-associated T-cell lymphoma.[115] These patients typically undergo pharmacologic therapies, including treatment with steroids[116,117] or immunosuppressants, such as azathioprine[118] and cyclosporine.[119] If patients do not respond to these treatments, the ultimate treatment is total parenteral nutrition. None of these therapies have been subjected to rigorous controlled studies.[120]

In young children with villus atrophy whose symptoms do not respond to a gluten-free diet, diseases that must be considered include tufting enteropathy and other congenital ultrastructural abnormalities of the enterocyte,[121] unrecognized chronic giardiasis, and autoimmune enteropathy.

For a more comprehensive overview on refractory sprue, the reader is referred to a recently published review.[122]

Future Directions

A multidisciplinary research effort to understand the pathogenesis of CD is currently taking place worldwide. This effort is fueled by the appreciation that CD represents a unique example of an autoimmune disease in which the environmental factor(s) that induce the immune response has been identified. Therefore, scientists view CD as a model to tackle key questions on the pathogenic mechanisms involved in other autoimmune diseases (i.e., multiple sceloris, diabetes mellitus, rheumatoid arthritis, etc.) whose environmental triggers are still unknown. Future directions in CD research (Table 5) have been clearly identified and were recently discussed both at the 9th International Symposium on CD that was held on August 10–13 in Baltimore[123] and at the first World Congress of Pediatric Gastroenterology, Hepatology, and Nutrition in Boston.[124] Although some of these goals are in an advanced state of development (i.e., engineering gluten-free grains), others (i.e., the search for the CD genes) are extremely challenging and will require an international task force to generate meaningful data. Nevertheless, the appreciation that CD is not a disease confined in Europe but a global problem affecting continents such as North and South America, Africa, and Asia, where it was historically considered an extremely rare condition, is catalyzing the scientific attention of new generations of investigators who will surely help achieve these challenging targets.

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