Coeliac disease

Benjamin Lebwohl, David S Sanders, Peter H R Green

Coeliac disease occurs in about 1% of people in most populations. Diagnosis rates are increasing, and this seems to be due to a true rise in incidence rather than increased awareness and detection. Coeliac disease develops in genetically susceptible individuals who, in response to unknown environmental factors, develop an immune response that is subsequently triggered by the ingestion of gluten. The disease has many clinical manifestations, ranging from severe malabsorption to minimally symptomatic or non-symptomatic presentations. Diagnosis requires the presence of duodenal villous atrophy, and most patients have circulating antibodies against tissue transglutaminase; in children, European guidelines allow a diagnosis without a duodenal biopsy provided that strict symptomatic and serological criteria are met. Although a gluten-free diet is an effective treatment in most individuals, a substantial minority develop persistent or recurrent symptoms. Difficulties adhering to a gluten-free diet have led to the development of non-dietary therapies, several of which are undergoing trials in human beings.

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Celiac Disease Center, College of Physicians and Surgeons (B Lebwohl MD, Prof P H R Green MD), **and Department of Epidemiology, Mailman School of Public Health** (B Lebwohl), **Columbia University Medical Center, New York, NY, USA; and Academic Unit of Gastroenterology, Royal Hallamshire Hospital & University of Sheffield, UK** (Prof D S Sanders MD)

Correspondence to: Prof Peter H R Green, Celiac Disease Center, Columbia University, New York, NY 10032, USA
pg11@columbia.edu

Introduction

Coeliac disease is an autoimmune disorder that occurs in genetically predisposed individuals who develop an immune reaction to gluten. The disease primarily affects the small intestine; however, the clinical manifestations are broad, with both intestinal and extra-intestinal symptoms. Coeliac disease is notable because of its broad clinical spectrum of presentations, large age range at which onset can occur (which can precede diagnosis by several years), and the increased morbidity and mortality that has been found in most studies. The disease also provides a model of an immune-based disease with both strong genetic and environmental risk factors. Coeliac disease has been the topic of previous Seminars in *The Lancet*,[1,2] and we aim to provide the most current information to clinicians with this Seminar.

Pathogenesis

Gluten

The major environmental factor responsible for the development of coeliac disease is gluten. Gluten (from the Latin "glue") is the term for the prolamin storage proteins of the cereal grains wheat, rye, and barley. Gluten is favoured in breadmaking for its elasticity; however, it is enriched in glutamines and prolines and, as a result, is incompletely digested by gastric, pancreatic, and brush border peptidases, leaving large peptides up to 33 aminoacids long.[3] These peptides enter the lamina propria of the small intestine via transcellular or paracellular routes[4–6] where, in affected individuals, an adaptive immune reaction occurs that is dependent on deamidation of gliadin molecules by the enzyme tissue transglutaminase (TTG), the predominant autoantigen of coeliac disease.[7] Deamidation increases the immunogenicity of gliadin, facilitating binding to the HLA-DQ2 or HLA-DQ8 molecules on antigen presenting cells.[8] Gliadin peptides are then presented to gliadin-reactive CD4+ T cells.[9] During this process, antibodies against TTG, gliadin, and actin are made through unclear mechanisms. These antibodies might contribute to extra-intestinal manifestations of coeliac disease, such as dermatitis herpetiformis and gluten ataxia.[10,11]

Accompanying this adaptive immune reaction is an innate immune response in the epithelial compartment,[12] which is evident pathologically by prominent intraepithelial lymphocytosis. During the pathogenesis of coeliac disease, intraepithelial lymphocytes express the natural killer T-lymphocyte receptors NKG2D and CD9/NKG2A, which recognise the products (cell surface glycoproteins) of stress-induced genes *MICA* and *MICB* and the protein HLA-E expressed on the surface of epithelial cells. Interleukin 15 serves an important role in upregulating these natural killer receptors on cytotoxic epithelial cells.[13] Both the lamina propria (adaptive) and intraepithelial (innate) immune responses seem to be necessary for formation of the complete coeliac pathological lesion, but how these two processes interact is not clear.

People with coeliac disease develop an intense immune response to some but, notably, not all non-gluten proteins in wheat.[14] The importance of these non-gluten wheat proteins in the pathogenesis of coeliac disease is not clear, although one class of these proteins, the amylase trypsin inhibitors, might have a role in the epithelial cell damage, resulting from the innate response and in non-coeliac gluten sensitivity, another wheat-related disorder.[15,16]

Genetic factors

The importance of a genetic component for the development of coeliac disease is evident, based on the familial occurrence and the high concordance among

Search strategy and selection criteria

We searched PubMed for articles published from Jan 1, 1990, to Jan 31, 2017, with no language restrictions, using the terms "celiac", "coeliac", and "gluten". In our selection of articles, we emphasised those published since 2010, but included older publications of scientific and historical relevance. We mostly selected cohort and case-control studies and the few randomised trials performed in this subject area, but also selected guidelines and systematic reviews and smaller, non-controlled clinical studies of particular relevance.

identical twins.[17,18] Almost 100% of patients with coeliac disease possess specific variants of the HLA class II genes *HLA-DQA1* and *HLA-DQB1* that, together, encode the two chains (α and β) of the coeliac-associated heterodimer proteins DQ2 and DQ8 that are expressed on the surface of antigen presenting cells. More than 90% of patients with coeliac disease are DQ2 positive and most of the others are DQ8 positive. Geographical variability in the prevalence of DQ2 and DQ8 among patients with coeliac disease has been reported.[19] Some individuals with coeliac disease do not have the full component of alleles that compose the haplotype HLA-DQ2 and are, therefore, DQ2 negative, but are considered half DQ2 positive.[20] This finding indicates the importance for the clinician that the reports of whether a patient is HLA-DQ2 positive or HLA-DQ8 positive should include not only whether the haplotypes are present, but also whether the allelic components are present. A cohort study of children in Denver (CO, USA)[21] found that, by age 15 years, an estimated 3·1% of the population develops coeliac disease, with a risk of 14·2% among those homozygous for DQ2 and 1·5% among those who had one copy of DQ8.

Almost all patients with coeliac disease possess HLA-DQ2, HLA-DQ8, or half HLA-DQ2; however, up to 40% of people in the Americas, Europe, and southeast Asia also carry these alleles, indicating that these genes are necessary but not sufficient for coeliac disease to develop, contributing to only about 40% of the genetic risk for coeliac disease. Genome-wide association studies have shown 39 non-HLA regions associated with increased risk for coeliac disease. Demonstration of other genetic risk factors within and outside the MHC region that are associated with increased risk of coeliac disease allows for identification of possible pathogenic pathways, providing insight into pathogenic mechanisms.[22,23]

Environmental factors

The prerequisite HLA genes and gluten ingestion are common; however, coeliac disease occurs only in about 1% of the population, suggesting that other environmental factors besides gluten are probably important.

Breastfeeding and infant feeding practices

The Swedish epidemic of coeliac disease between 1984 and 1996 was considered to be the result of changes in infant feeding practices.[24] However, studies have not shown an effect of breastfeeding on the risk of coeliac disease.[25] Observational and large prospective studies of gluten introduction in children who were at high risk of coeliac disease due to a family history and compatible HLA haplotype did not show that the timing of gluten introduction had a significant effect on risk of coeliac disease.[26–28] Although delaying gluten introduction beyond 12 months of age can result in a lower risk of coeliac disease in the short term, this seems to be negated by a catch-up phenomenon later in childhood.[27] In a prospective cohort study, the most prominent risk factor for developing coeliac disease was the dosage of the HLA risk genes.[29] Despite the negative results of studies testing various strategies of the timing of gluten introduction, a high quantity of gluten remains a proposed risk factor, based on the results of a nested case-control study that showed that children with coeliac disease were consuming greater quantities of gluten compared with controls.[30] However, this result was not observed in a re-evaluation of another large study of similarly at-risk children who developed coeliac disease.[31] Because of the negative clinical trials, the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition issued modified guidelines regarding gluten introduction, acknowledging the absence of effective prevention strategies. These guidelines recommend that gluten be introduced between the ages of 4 months and 12 months, and that consumption of large quantities of gluten should be avoided during the first weeks after gluten introduction.[32] A meta-analysis showed that the late introduction of gluten (>6 months) increased the risk of developing coeliac disease.[33]

Other risk factors

Season of birth[34,35] and elective caesarean section[36,37] are risk factors for development of the disease, although studies of elective caesarean sections have shown conflicting results. Gastrointestinal infections,[38] rotavirus in children and campylobacter infection in adults, have been reported as risk factors,[39,40] with rotavirus vaccination seeming to provide a protective effect.[41] An increased total number of infections (>10 *vs* <4 during the first 18 months of life) and respiratory infections (during the first 18 months of life) seem to increase the risk of developing coeliac disease later in childhood.[42] Antibiotic[43] and proton pump inhibitor use[44] have been associated with increased risk of subsequent coeliac disease, as has in utero exposure to maternal iron supplementation.[45] However, in utero exposure to antibiotics has not been associated with coeliac disease risk.[46] *Helicobacter pylori* colonisation might decrease the risk of coeliac disease.[47] Infection with the otherwise non-pathogenic reovirus might trigger coeliac disease.[48]

Role of the microbiome

The complex interaction between genes, diet, and the microbiome might be crucial to the development of coeliac disease and to the generation of potential preventive or therapeutic measures. A study[49] in mice expressing HLA-DQ8 showed that the intestinal microbiota can enhance or attentuate gluten-induced immunopathology, dependent on the specific microbial milieu. Cross-sectional studies have shown that patients with coeliac disease have alterations to their intestinal microbiome that are not entirely normalised following introduction of a gluten-free diet. Faecal concentrations

of *Bifidobacterium bifidum* were found to be significantly higher in untreated patients with coeliac disease than in healthy adults,[50] and children with coeliac disease had a higher incidence of duodenal Gram-negative and, potentially, pro-inflammatory bacteria at diagnosis than did children in a control group.[51] Another study[52] showed that, although bacterial diversity was higher in non-coeliac controls than in untreated patients with coeliac disease, the difference was not significant. Age and a gluten-free diet can affect the duodenal microbiome in patients with coeliac disease,[53] but a gluten-free diet will alter the microbiome even in healthy individuals.[54] A 2015 study[55] showed specific alterations in the faecal bacteria of vaginally delivered, breastfed infants at risk for coeliac disease that was associated with presence of HLA-DQ2, indicating that HLA type selects for specific gut microbiome characteristics.

Epidemiology

Coeliac disease affects about 1% of the population.[56,57] Worldwide, there are differences in prevalence that are not explained by the known genetic and environmental risk factors. For example, in Europe, Germany has a lower prevalence of coeliac disease than other countries, with the highest prevalence being in Sweden and Finland.[58] Within the USA, the prevalence in African Americans is low compared with those of white ethnic background;[59] similarly, in Brazil, Brazilians of African descent have low rates of coeliac disease.[60] The disease is increasingly prevalent in India, with the highest prevalence in northern India; this region has a similar prevalence of compatible HLA haplotypes compared with other regions in India, but has a much higher rate of wheat consumption.[61] A 2016 study[62] of nearly 500 000 duodenal biopsy samples taken from people throughout the USA showed that the ethnic group (based on a name-based algorithm) with the highest prevalence of villous atrophy was among those originally descended from the Punjab area of northern India (3·08% *vs* 1·80% for other Americans).

Globally, the prevalence of coeliac disease is increasing. Studies comparing serum stored from 1948–54 to current serum samples from the USA showed an increasing prevalence with time: a 4–4·5 times increase over approximately 50 years (comparing samples from 1948–54 to the present day)[63] and a 2 times increase over 15 years (between 1974 and 1989).[64] Similarly, in Finland, a 2 times increase in prevalence has been shown over approximately 20 years, comparing serum from 1978–80 to 2000–01.[65] A study[21] done in Denver, CO, USA followed children with increased genetic risk of coeliac disease, during a 20-year period; when these findings were extrapolated to the general population of the city, the cumulative incidence values for coeliac disease were 1·6% at 5 years of age, 2·8% at 10 years, and 3·1% at 15 years, a notable incidence rate which is similar to that seen in Scandinavians.[66] These estimates are substantially higher than those of 2016 population-based estimates of coeliac disease prevalence,[67] which suggested a prevalence of less than 1% of adults and children in the USA.

While the prevalence of coeliac disease has increased, the rate of diagnosis has increased more slowly.[68] Analysis of the National Health and Nutrition Examination Survey, a national survey that includes about 5000 individuals annually and is considered representative of the US population, showed that more than 80% of people with coeliac disease were undiagnosed in 2009 and this decreased to less than 50% in 2013–14.[67] If these numbers are confirmed in subsequent studies, this would indicate that, in the USA, there has been a large increase in the rate of diagnoses of coeliac disease similar to that seen previously in Finland, where the increase had been attributed to a rise in physician education.[69] The increase in diagnosis rate in the USA could also be partly because of increased general interest in and adoption of the gluten-free diet in the general population in recent years.[67]

Clinical manifestations

Over the past 10 years, attempts have been made to bring consensus to the terminology of the clinical stages of coeliac disease (panel 1).[70] The common presentation of coeliac disease has shifted from the historically classic symptoms of malabsorption in childhood to non-classic symptoms, which can be present in childhood or adulthood. Classic symptoms include chronic diarrhoea, weight loss, and failure to thrive,[71] which are quite rare. The more common, non-classical symptoms include iron deficiency, bloating, constipation, chronic fatigue, headache, abdominal pain, and osteoporosis. A 2010 study[72] showed a considerably impaired quality of life in patients living with undiagnosed coeliac disease compared with those who had been diagnosed and treated (EuroQol five dimensions questionnaire score 0·56 *vs* 0·84). Increased awareness is required in both primary and secondary care to recognise the shift of the common presenting features and the non-specific manifestations of coeliac disease. Furthermore, patients can present with these diverse features to many different sub-specialties of medicine. Coeliac UK (the UK national patient body) report an average 13-year delay in diagnosis for patients. As a result, in 2015, the National Institute for Health and Care Excellence (NICE) updated their guidelines for coeliac disease and created a list of symptoms or risk factors that should trigger testing for coeliac disease (panel 2).[71]

For more on **Coeliac UK** see https://www.coeliac.org.uk/home

Diagnosis

A combination of coeliac disease serology testing and duodenal biopsy sampling is required for the diagnosis of coeliac disease in adults. The current American College of Gastroenterology,[73] British Society of Gastroenterology,[74] and NICE guidelines[71] recommend testing high-risk adults with coeliac serology. Measurement of the concentration of IgA-TTG antibodies

Panel 1: **Terminology describing patients with coeliac disease (adapted from Ludvigsson and colleagues, 2013)[70]**

Potential
Positive serological tests and normal intestinal biopsy

Asymptomatic
Absence of symptoms despite specific questioning regarding symptoms

Symptomatic
Presence of either intestinal or extra-intestinal symptoms

Classic
Diarrhoea, signs and symptoms of malabsorption, or both

Non-classic
Lack of malabsorption symptoms, but other symptoms present (eg, anaemia, osteoporosis)

Refractory
Persistent symptoms and villous atrophy despite adherence to a gluten-free diet

Panel 2: **National Institute for Health and Care Excellence guidelines[71] on the indications that should prompt testing for coeliac disease**

Coeliac testing recommended
- Persistent unexplained abdominal or gastrointestinal symptoms
- Faltering growth
- Prolonged fatigue
- Unexpected weight loss
- Severe or persistent mouth ulcers
- Unexplained iron, vitamin B12, or folate deficiency
- Type 1 diabetes
- Autoimmune thyroid disease
- Irritable bowel syndrome
- First degree relatives of people with coeliac disease

Coeliac testing should be considered
- Metabolic bone disorders (reduced bone mineral density or osteomalacia)
- Unexplained neurological symptoms (particularly peripheral neuropathy or ataxia)
- Unexplained subfertility or recurrent miscarriage
- Persistently increased concentrations of liver enzymes with unknown cause
- Dental enamel defects
- Down's syndrome
- Turner syndrome

should be done as a first-line screening test because of its high sensitivity and negative predictive value, and because it is less expensive than measurement of endomysial antibodies (EMA). A study of 2000 patients[75] (with a 3·4% prevalence of coeliac disease) compared the sensitivities of IgA TTG tests alone and a two-step approach using IgA TTG and EMA testing. IgA TTG testing alone was found to be a more sensitive marker for coeliac disease than IgA-TTG plus EMA testing (sensitivities of 90·9% *vs* 85·7%, and negative predictive values of 99·6% *vs* 99·7%). Therefore, IgA-TTG testing is the recommended first-line approach. However, if IgA-TTG is weakly positive (compared with the laboratory threshold of normal), EMA concentration, which has a high specificity (95%), should also be tested,[74,76] Notably, EMA testing uses the substrates of monkey oesophagus or human umbilicus, which have low availability. EMA testing is also expensive and labour intensive, and interpretation of the results can be subjective. Evidence suggests that detection of antibodies against deamidated gliadin peptide might allow recognition of some cases of coeliac disease that are not detected by the established serological tests,[77] although isolated increases in the concentration of this antibody (in the context of a normal TTG titre) have a low positive predictive value for coeliac disease.[78] Ultimately, diagnostic practice is likely to vary internationally depending on the available tests and the health-care system within each individual country.

Patients with IgA deficiency do not produce IgA-TTG or IgA-EMA antibodies, and could have a false negative result. Therefore, total IgA concentration should be measured in conjunction with serology. For patients with IgA deficiency, IgG-TTG, IgG-EMA, and IgG-deamidated gliadin peptide can be tested instead. A 2012 meta-analysis[79] showed that IgG-deamidated gliadin peptide had a pooled sensitivity ranging from 80·1% to 98·6%.

Advising patients to eat a gluten-containing diet before their tests to ensure the serological and histological results are not affected is important. Although the minimum intake of gluten required for diagnosis has historically been 10 g of gluten a day (equivalent to four slices of bread) for 6 weeks, more recent data[80,81] showed that a shorter, lower dose of gluten challenge could be sufficient to induce serological and histological changes in patients with coeliac disease (>3 g gluten per day for 2 weeks or 10 g gluten per day for 18 days). In the 3 g per day gluten challenge, 68% of patients developed villous atrophy within 14 days.[80] In this study, the concentration of serological markers for coeliac disease increased at a slower rate than did histological abnormalities (table). The decision of whether to proceed with a duodenal biopsy during a gluten challenge should depend on the pre-test likelihood of coeliac disease. For example, if the likelihood is low, negative serological tests following 28 days of a low-dose gluten-challenge are sufficient to rule out coeliac disease with high confidence. If the likelihood is high, a duodenal biopsy sample should be done at least 14 days after beginning the gluten challenge, because of the possibility of seronegative coeliac disease.

Duodenal biopsy samples showing increased intra-epithelial lymphocytes, crypt hyperplasia, and villous

atrophy (Marsh type 3) with positive coeliac serology confirm the diagnosis of coeliac disease in adults. Biopsy sampling is done during gastroscopy, and abnormalities such as loss of folds or scalloping of folds in the descending duodenum might be observed;[82] however, the duodenum can appear normal (and these changes can be seen in other conditions, such as giardiasis and infection with HIV).[83,84] Although IgA-TTG and EMA have excellent sensitivities in the medical literature,[76] their sensitivities often decrease when the tests are done in real-world health-care settings, with a lower prevalence of coeliac disease being reported in the general population than in prospective studies. Therefore, a confirmatory duodenal biopsy sample ensures that patients are correctly diagnosed with coeliac disease before being subjected to a gluten-free diet for life. Additionally, this confirmatory biopsy avoids any diagnostic uncertainty in cases where patients do not respond to a gluten-free diet, and provides a baseline reference for histological response to a gluten-free diet. Notably, a clinical response to a gluten-free diet alone does not confer a diagnosis of coeliac disease. This clinical response can also be seen in patients with non-coeliac gluten sensitivity, which is characterised by symptom improvement on gluten avoidance without coeliac disease (with negative coeliac serology and absence of villous atrophy).[85] Negative serological findings do not exclude coeliac disease with 100% accuracy, because of the possibility of seronegative coeliac disease.

Paediatric practice has moved towards a so-called biopsy avoidance strategy. The 2012 European Society for Paediatric Gastroenterology, Hepatology, and Nutrition guidelines[86] recommend that symptomatic paediatric patients no longer require biopsy sampling to confirm the diagnosis of coeliac disease if they have TTG concentrations 10 times higher than the upper limit of normal, positive EMA in a separate blood sample, and the presence of HLA-DQ2 or HLA-DQ8 genotype. The rationale for requiring a second blood sample is: (1) to prevent the possibility of a mislabelled sample leading to an erroneous diagnosis; and (2) to detect the possibility of temporary gluten autoimmunity, which is not uncommon.[21] This non-biopsy strategy avoids the need for a gastroscopy, which often requires general anaesthesia in paediatric patients. This is an appropriate approach in carefully selected symptomatic paediatric cases, as judged by specialists. Practice of this strategy has led to questions of the usefulness of biopsy avoidance in adults, since data show a similarly high positive predictive value of TTG concentrations that are increased to this degree,[87] and further data suggest that discrepancies occur in the interpretation of duodenal histopathology in the absence of formal morphometric measurement.[88] However, it is premature to conclude that this strategy should be extended to adult practice. With the variable specificities of TTG assays across the UK and the USA, adults who do not have coeliac disease risk being committed to a gluten-free diet.

	Day 14	Day 28
Villous atrophy	68·4%	..
Increased tissue transglutaminase IgA, deamidated gliadin peptide IgA, or deamidated gliadin peptide IgG	50·0%	89·5%

Data are from 19 patients who are undergoing gluten challenge. Adapted from Leffler and colleagues.[80]

Table: **Development of abnormalities during gluten challenge**

The histological changes associated with coeliac disease are patchy—villous atrophy can be present in areas that are adjacent to non-atrophic villi. To optimise the likelihood of a histological diagnosis, a specific duodenal biopsy sample strategy should be used. New evidence supports the usefulness of a duodenal bulb biopsy in diagnosis of coeliac disease and requires a minimum of four further biopsy samples from the second part of the duodenum. For the bulb biopsy, no specific anatomical site within the bulb is required.[89] Although inclusion of bulb biopsies has 100% sensitivity in the recognition of villous atrophy,[89] in a low pre-test probability setting, the incremental yield of bulb biopsies is low, and raises the possibility of other pathological abnormalities being misinterpreted as coeliac disease.[90] The bulb biopsy could, therefore, require a separate formalin specimen to allow the pathologist to appropriately interpret the different duodenal anatomy, such as the presence of Brunner's glands, heterotopic gastric mucosa, or lower baseline intraepithelial lymphocyte counts. Furthermore, a so-called single-bite biopsy technique has better histological orientation (in which crypt-to-villus ratios can be adequately measured) than do so-called double-bite biopsies (66% for the single-biopsy technique compared with 42% of patients for the double-biopsy technique; $p<0\cdot01$).[91] Despite guidelines,[73,74] current real-world adherence to best clinical practice in health-care settings is still highly variable and is likely to further contribute to delays in diagnosis.[92] Current medical literature suggests that between 5% and 13% of patients with diagnosed coeliac disease have had a previous endoscopy with either no biopsy or an inadequate biopsy resulting in a delay in diagnosis.[93,94]

Other diagnostic challenges exist: HLA genotyping, although not required as a routine test in all patients with suspected coeliac disease, can be valuable in equivocal diagnoses, patients who are already on a gluten-free diet who are unwilling or unable to undergo a gluten challenge, and those who refuse a gastroscopy.[95] HLA testing can also be useful when assessing family members of patients with coeliac disease, because the absence of HLA-DQ2 and HLA-DQ8 almost always excludes coeliac disease, with a negative predictive value of more than 99%. However, the allelic components should be noted, because patients with a half-DQ2 result can develop coeliac disease, although this finding is rare. Among patients who are unwilling or unable to undergo gastroscopy, video capsule

endoscopy can also provide helpful information, because mucosal changes indicative of coeliac disease can be identified.[96,97]

Individuals with seronegative duodenal villous atrophy can have an alternative cause of this histological abnormality than coeliac disease. However, a subset of patients (between 2% and 15% of the population of patients with coeliac disease) with seronegative villous atrophy have seronegative coeliac disease.[98–100] Seronegative coeliac disease can result from impaired immunoregulation such as IgA deficiency, concomitant common variable immunodeficiency, and use of immunosuppressant. Seronegative coeliac disease can also occur early in coeliac disease development,[101] late in the disease (presenting as enteropathy-associated T-cell lymphoma), or in patients who have adopted a reduced-gluten diet before testing. Volta and colleagues[100] compared the clinical characteristics of seropositive and seronegative coeliac disease (n=810 patients), and reported that patients with seronegative coeliac disease were older at diagnosis (49 years *vs* 36 years; p<0·005) and had typical symptoms, such as diarrhoea, significantly more frequently (100% *vs* 34% of patients; p<0·001). Seronegative coeliac disease was also associated with more severe villous atrophy (67% *vs* 36% of patients) and coexisting autoimmune diseases than seropositive coeliac disease. These findings support those reported by Salmi and colleagues,[99] who found transglutaminase 2 autoantibodies deposited in the small bowel mucosa in patients with seronegative coeliac disease, despite their seronegativity. The authors indicated that, in seronegative coeliac disease, coeliac antibodies are bound to intestinal transglutaminase 2 autoantibodies with considerably high avidity, which occurs in a chronic immune reaction, rendering the antibodies unable to enter the circulation to cause seropositivity. Seronegative coeliac disease is, therefore, speculated to be associated with chronic, more severe forms of coeliac disease.

Seronegative coeliac disease requires careful diagnosis, with the presence of HLA-DQ2 or HLA-DQ8 and a response to a gluten-free diet after excluding other causes of seronegative villous atrophy. A large prospective analysis of 200 cases of seronegative villous atrophy[102] showed that 31% of cases were due to coeliac disease, 27% were caused by infection, and 18% were idiopathic, of which 72% spontaneously resolved without any intervention. Notably, non-white ethnicity was markedly associated with seronegative villous atrophy (odds ratio 10·8; p=0·003), and 66% of non-white cases of seronegative villous atrophy were caused by infection. Other causes of seronegative villous atrophy included use of specific medications (mycophenolate mofetil and olmesartan medoxomil), common variable immune deficiency, Crohn's disease, tropical sprue, giardiasis, autoimmune enteropathy, and other, less common diseases.[103]

Controversies in testing for coeliac disease

There is controversy as to whom to test for coeliac disease. A central tenet of this issue is the ethical difference between population screening and case-finding. If a patient seeks medical help then the physician is attempting to diagnose an underlying condition; for example, patients with coeliac disease can present with symptoms of irritable bowel syndrome or with osteoporosis. This behaviour would be classified as case-finding and, evidently, the patient has initiated the consultation and, in some sense, is consenting for investigation.

The Olmsted County Population database[57] reviewed 338 previously undetected individuals with coeliac disease (based on paired serology) from a population of 31255 residents of Olmsted County (MN, USA) whose sera had been collected and tested. On this basis, increased mortality or morbidity in those individuals with undiagnosed coeliac disease was not shown.[57,104] These data lend support to a no-screening policy for coeliac disease, although an alternative interpretation is that coeliac disease can be identified in a pre-symptomatic phase, providing proof-of-concept to the approach of early intervention. However, data to support that such an intervention leads to improved outcomes compared with a no-screening policy are lacking. Further controversy exists in the outcomes for patients with type 1 diabetes who are diagnosed with coexisting coeliac disease through screening programmes, because of a paucity of prospective longitudinal follow-up data.[105] A randomised trial assessing the effect of diagnosing and treating coeliac disease on glycaemic control and other outcomes in patients with type 1 diabetes is in progress.[106]

Treatment

The mainstay of treatment of coeliac disease remains adherence to a gluten-free diet. Improvement and resolution of symptoms typically occurs within days or weeks, and often precedes normalisation of serological markers and of duodenal villous atrophy.[107] Despite its effectiveness in achieving normalisation of these parameters in most patients, the gluten-free diet has numerous difficulties. Gluten-free substitute foods are substantially more expensive than their gluten-containing counterparts.[108] Patients with low incomes might, therefore, be at particularly high risk of non-adherence to this diet.[109] The quality of information regarding the gluten-free status of food ingredients is variable in online resources, which can lead to confusion among patients.[110] Potential gluten exposure when travelling or eating in restaurants can be a hazard and a source of anxiety.[111] Social pressures, particularly in adolescence, can also be an impediment to strict adherence.[112] Uncertainty regarding the presence of gluten in trace amounts in medications and supplements is another concern.[113] As a result of the vigilance required to adhere to this diet, the burden of treatment of coeliac

disease is high. The self-rated burden of treatment in coeliac disease in adults is greater than that rated by patients with chronic conditions such as hypertension, and is similar to the treatment burden of diabetes.[109]

Patients with newly diagnosed coeliac disease should be referred to an expert dietitian, because the gluten-free diet requires knowledge not only of hidden sources of gluten, but also of healthy gluten-free substitute grains that provide adequate fibre and nutrients. Upon diagnosis, patients should be tested for micronutrient deficiencies, including iron, folic acid, vitamin B12, and vitamin D.[73] Pneumococcal vaccination can be considered, because of the association between coeliac disease and increased risk of community-acquired pneumonia.[114] In view of the increased risk of osteoporosis and fractures in patients with coeliac disease,[115] guidelines issued by the British Society of Gastroenterology[74] recommend measurement of bone mineral density after 1 year of the gluten-free diet in patients older than 55 years or in those with additional risk factors for osteoporosis. Beyond the initial diagnosis period, patients should be followed up regularly for assessment of symptoms and to monitor adherence to the gluten-free diet, noting a combination of symptoms, serologies (which usually normalise within 1 year of starting the diet), and dietitian follow-up.[73]

Non-dietary therapies

Many patients with coeliac disease are not satisfied with the gluten-free diet and are interested in alternative, non-dietary therapies.[116] Together with the knowledge of the pathophysiological mechanism of coeliac disease, this finding has led to an interest in drug development either as an addition to the gluten-free diet or as a substitute.[117] Drugs in various stages of development and testing use mechanisms such as inactivation of the toxic peptides in the bowel lumen, prevention of passage of gliadin into the mucosa, induction of immune tolerance, and inactivation of the immune process in the lamina propria. Related to the inactivation of the immune process in the lamina propria, a vaccine consisting of epitopes for gluten-specific CD4-positive T cells has completed phase 1 clinical studies.[118]

Two drugs have progressed through phase 2 clinical studies. Larazotide acetate, an oral peptide that modulates tight junctions and prevents passage of gliadin peptides through the epithelial barrier, was superior to placebo in alleviating symptoms in patients on a gluten-free diet compared with the diet plus placebo in a 12-week study.[119] Latiglutenase, an enzyme preparation that prevents the pathological damage caused by gluten in patients with coeliac disease, was studied in a large clinical trial of patients with coeliac disease with symptoms and evidence of pathological damage (consistent with ongoing gluten ingestion despite attempts at adhering to the gluten-free diet).[120] Latiglutenase did no better than placebo in alleviating symptoms or villous atrophy, which was thought to be due to a trial effect in which patients in the placebo group became more compliant to the diet and reduced their gluten consumption. Further trials of both drugs are planned.

Non-responsive and refractory coeliac disease

About 20% of patients with coeliac disease have persistent or recurrent symptoms despite a gluten-free diet.[121] These cases are caused by heterogeneous conditions (panel 3). An essential first step in assessing these patients is to confirm the accuracy of the initial diagnosis of coeliac disease. If a patient did not originally have a duodenal biopsy showing villous atrophy while on a gluten-containing diet, or if the patient had a negative coeliac disease result from serological testing despite the presence of villous atrophy,[101,103] an alternative diagnosis is possible. In this context, revisiting the diagnosis of coeliac disease with HLA testing and a gluten challenge might be appropriate.

When the diagnosis of coeliac disease is confirmed, the most common cause of persistent symptoms is inadvertent gluten exposure, and this can be ascertained with careful assessment by a knowledgeable dietitian. Other causes of persistent symptoms include irritable bowel syndrome, microscopic colitis, lactose or fructose intolerance, pancreatic insufficiency, and small intestinal bacterial overgrowth.[121,122]

Patients with or without ongoing or recurrent symptoms can show persistent villous atrophy on follow-up. Although this seems to be more common in adults older than age 50 years,[123] villous atrophy persisted in 19% of children who underwent follow-up biopsy at least 1 year after starting the gluten-free diet.[124] In adults, persistent villous atrophy has been associated with an increased risk of osteoporotic fractures and lymphoproliferative malignancy, suggesting that mucosal healing could be an endpoint in assessment of the response to the gluten-free diet.[125,126] However, in view of the uncertainty regarding the degree of causation by persistent villous atrophy of these outcomes, and the absence of randomised trial data showing the effectiveness of routine follow-up biopsy, guidelines[73,74] reassessment of duodenal histology can be considered, as opposed to routinely recommended. The finding of persistent villous atrophy could lead to implementation of successful strategies that have previously been evaluated, such as more intensive dietitian follow-up,[127] or the short-term adoption of a more stringent diet eliminating all processed foods.[128]

Refractory coeliac disease is diagnosed in patients with persistent or recurrent symptoms of malabsorption and villous atrophy, despite evidence of strict gluten avoidance. This condition is associated with increased mortality and can progress to enteropathy-associated T-cell lymphoma. Patients with suspected refractory coeliac disease should have a duodenal biopsy, with assessment for an aberrant intraepithelial T-cell

For more on the **drugs in development** see www.clinicaltrials.gov/ct2/results?term=celiac+disease&Search=Search

Panel 3: Causes of non-responsive coeliac disease

Incorrect initial diagnosis
- Non-coeliac gluten sensitivity
- Seronegative villous atrophy

Inadvertent gluten exposure

Additional conditions
- Irritable bowel syndrome
- Small intestinal bacterial overgrowth
- Food intolerance (eg, to lactose or fructose)
- Pancreatic exocrine insufficiency
- Microscopic colitis

Refractory coeliac disease

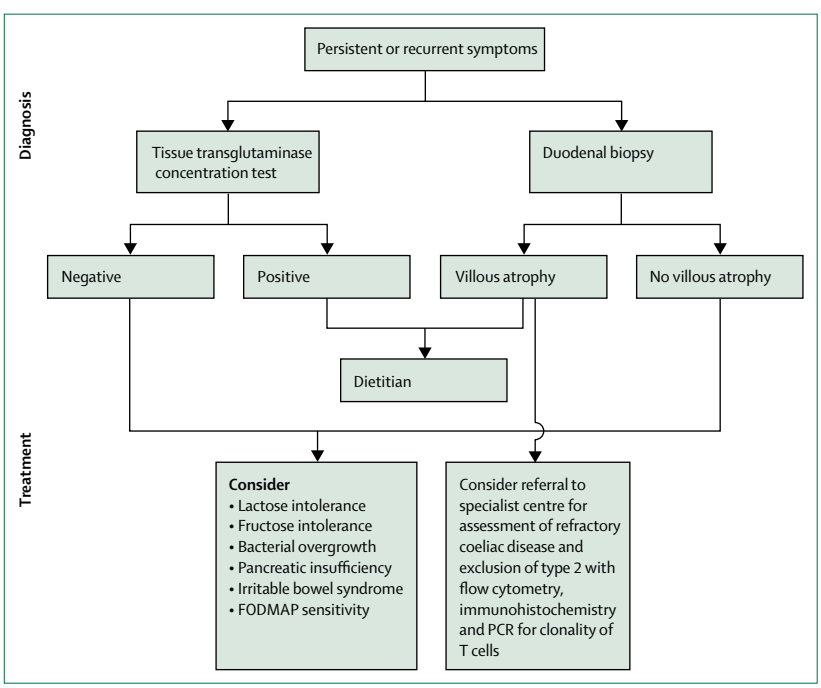

Figure: **Proposed flowchart for diagnosis and treatment following persistent or recurrent symptoms in patients with coeliac disease**

population using immunohistochemistry, flow cytometry, and PCR for detection of T-cell receptor γ (TCR γ) gene rearrangement. The aberrant intraepithelial lymphocytes in patients with type 2 refractory coeliac disease lack surface CD8, CD3, and TCR γ expression while they express intracytoplasmic CD3, and exhibit oligoclonal TCR gene rearrangement.[129] Those patients without an aberrant T-cell population (type 1 refractory coeliac disease) have a better prognosis than do those with aberrant T cells (type 2 refractory coeliac disease), and those with type 2 refractory coeliac disease are at high risk for progression to enteropathy-associated T-cell lymphoma.[130] A three-item clinical score incorporating age, serum albumin concentration, and the presence of aberrant lymphocytes predicts 5-year survival in patients with refractory coeliac disease.[131] We have generated a proposed flowchart for the diagnosis and treatment following persistent or recurrent symptoms in patients with coeliac disease (figure).

Malignancy and mortality risk

In addition to enteropathy-associated T-cell lymphoma, coeliac disease is associated with an increase in other types of non-Hodgkin lymphoma.[132,133] In a population-based study, the risk of increased malignancy was increased for adenocarcinoma of the oesophagus, small intestine, colon, liver, and pancreas, although only estimates for the small intestine and liver remained significant after excluding the first year after coeliac disease diagnosis.[134] The risk of colorectal cancer was found to be modestly increased in the long term (>5 years after diagnosis) in a second population-based study,[133] but these patients did not seem to have an increased prevalence of colorectal adenomas.[135] The risks of breast and lung carcinoma are reduced in patients with coeliac disease,[133,136] but this might be because smoking is less common in patients with coeliac disease.[137] In view of these differential associations of coeliac disease with malignancy risk according to organ type, pooling overall malignancy as an outcome yielded a null association with coeliac disease in a 2012 meta-analysis.[138]

An association between coeliac disease and increased mortality is well documented, with several studies[139,140] showing an increased risk of mortality that is reduced with time after diagnosis of coeliac disease. A population-based study in Sweden[140] investigating cause-specific mortality found that patients with coeliac disease were at increased risk of death due to cardiovascular disease, pulmonary disease, and cancer. The mortality risk associated with undiagnosed coeliac disease remains uncertain. Although a study found a large increase in mortality among people with undiagnosed coeliac disease who gave serum to the Warren Air Force Base (Cheyenne, WY) in the USA (hazard ratio 3·9),[63] other analyses of stored serum found no increase in mortality.[141,142] These contrasting findings could be due to differing definitions of coeliac disease or heterogeneous settings and time periods, in which the clinical threshold that would prompt a diagnosis could be varied. Although the data are weak, it is reasonable to conclude that coeliac disease, particularly symptomatic yet untreated coeliac disease, is associated with a modestly increased risk of mortality.

Conclusions

Despite the increase in the prevalence of coeliac disease and improved recognition and rates of diagnosis, numerous avenues of investigation are necessary to better understand the pathogenesis and improve the treatment of patients with this condition. Advances in the pathophysiology of coeliac disease could enable preventive strategies in individuals at high risk for disease development. The development of non-dietary

therapies might alleviate symptoms among patients with coeliac disease and inadvertent gluten exposure, and an effective replacement of the gluten-free diet could greatly enhance the quality of life of those many patients who find adhering to the diet challenging. Technologies for the detection of gluten in food and to monitor for recent gluten exposure (such as the detection of gluten peptides in stool or urine)[143,144] could enhance the design of future clinical trials and might be of major value in patients' daily activities.

Contributors

All authors contributed equally to the literature search and to writing the manuscript.

Declaration of interests

DSS receives an educational grant from Dr Schär, a gluten-free company, for an investigator-led study, but reports that this company have no control over outcomes. PHRG is on the scientific advisory board for ImmusanT, Celimmune, and ImmunogenX. BL declares no competing interests.

References

1 Green PH, Jabri B. Coeliac disease. *Lancet* 2003; **362:** 383–91.

2 Di Sabatino A, Corazza GR. Coeliac disease. *Lancet* 2009; **373:** 1480–93.

3 Shan L, Molberg Ø, Parrot I, et al. Structural basis for gluten intolerance in celiac sprue. *Science* 2002; **297:** 2275–79.

4 Matysiak-Budnik T, Moura IC, Arcos-Fajardo M, et al. Secretory IgA mediates retrotranscytosis of intact gliadin peptides via the transferrin receptor in celiac disease. *J Exp Med* 2008; **205:** 143–54.

5 Visser J, Rozing J, Sapone A, Lammers K, Fasano A. Tight junctions, intestinal permeability, and autoimmunity: celiac disease and type 1 diabetes paradigms. *Ann N Y Acad Sci* 2009; **1165**: 195–205.

6 Schumann M, Siegmund B, Schulzke JD, Fromm M. Celiac disease: role of the epithelial barrier. *Cell Mol Gastroenterol Hepatol* 2017; **3:** 150–62.

7 Dieterich W, Ehnis T, Bauer M, et al. Identification of tissue transglutaminase as the autoantigen of celiac disease. *Nat Med* 1997; **3:** 797–801.

8 Molberg O, Mcadam SN, Körner R, et al. Tissue transglutaminase selectively modifies gliadin peptides that are recognized by gut-derived T cells in celiac disease. *Nat Med* 1998; **4:** 713–17.

9 Lundin KE, Scott H, Hansen T, et al. Gliadin-specific, HLA-DQ(alpha 1*0501,beta 1*0201) restricted T cells isolated from the small intestinal mucosa of celiac disease patients. *J Exp Med* 1993; **178:** 187–96.

10 Taylor TB, Schmidt LA, Meyer LJ, Zone JJ. Transglutaminase 3 present in the IgA aggregates in dermatitis herpetiformis skin is enzymatically active and binds soluble fibrinogen. *J Invest Dermatol* 2015; **135:** 623–25.

11 Hadjivassiliou M, Aeschlimann P, Sanders DS, et al. Transglutaminase 6 antibodies in the diagnosis of gluten ataxia. *Neurology* 2013; **80:** 1740–45.

12 Abadie V, Discepolo V, Jabri B. Intraepithelial lymphocytes in celiac disease immunopathology. *Semin Immunopathol* 2012; **34:** 551–66.

13 Abadie V, Jabri B. IL-15: a central regulator of celiac disease immunopathology. *Immunol Rev* 2014; **260:** 221–34.

14 Huebener S, Tanaka CK, Uhde M, et al. Specific nongluten proteins of wheat are novel target antigens in celiac disease humoral response. *J Proteome Res* 2015; **14:** 503–11.

15 Junker Y, Zeissig S, Kim SJ, et al. Wheat amylase trypsin inhibitors drive intestinal inflammation via activation of toll-like receptor 4. *J Exp Med* 2012; **209:** 2395–408.

16 Gasbarrini G, Mangiola F. Wheat-related disorders: a broad spectrum of 'evolving' diseases. *United European Gastroenterol J* 2014; **2:** 254–62.

17 Kuja-Halkola R, Lebwohl B, Halfvarson J, Wijmenga C, Magnusson PK, Ludvigsson JF. Heritability of non-HLA genetics in coeliac disease: a population-based study in 107 000 twins. *Gut* 2016; **65:** 1793–98.

18 Fasano A, Berti I, Gerarduzzi T, et al. Prevalence of celiac disease in at-risk and not-at-risk groups in the United States: a large multicenter study. *Arch Intern Med* 2003; **163:** 286–92.

19 Karell K, Louka AS, Moodie SJ, et al. HLA types in celiac disease patients not carrying the DQA1*05-DQB1*02 (DQ2) heterodimer: results from the European Genetics Cluster on Celiac Disease. *Hum Immunol* 2003; **64:** 469–77.

20 Taylor AK, Lebwohl B, Snyder CL, et al. Celiac disease. In: Pagon RA, Adam MP, Ardinger HH, et al, eds. GeneReviews, Seattle: University of Washington, 2015.

21 Liu E, Dong F, Barón AE, et al. High incidence of celiac disease in a long-term study of adolescents with susceptibility genotypes. *Gastroenterology* 2017; **152:** 1329–36.

22 Gutierrez-Achury J, Zhernakova A, Pulit SL, et al. Fine mapping in the MHC region accounts for 18% additional genetic risk for celiac disease. *Nat Genet* 2015; **47:** 577–78.

23 Castellanos-Rubio A, Fernandez-Jimenez N, Kratchmarov R, et al. A long noncoding RNA associated with susceptibility to celiac disease. *Science* 2016; **352:** 91–95.

24 Ivarsson A, Persson LA, Nyström L, et al. Epidemic of coeliac disease in Swedish children. *Acta Paediatr* 2000; **89:** 165–71.

25 Welander A, Tjernberg AR, Montgomery SM, Ludvigsson J, Ludvigsson JF. Infectious disease and risk of later celiac disease in childhood. *Pediatrics* 2010; **125:** e530–36.

26 Aronsson CA, Lee HS, Liu E, et al, for the TEDDY Study Group. Age at gluten introduction and risk of celiac disease. *Pediatrics* 2015; **135:** 239–45.

27 Lionetti E, Castellaneta S, Francavilla R, et al, for the SIGENP (Italian Society of Pediatric Gastroenterology, Hepatology, and Nutrition) Working Group on Weaning and CD Risk. Introduction of gluten, HLA status, and the risk of celiac disease in children. *N Engl J Med* 2014; **371:** 1295–303.

28 Vriezinga SL, Auricchio R, Bravi E, et al. Randomized feeding intervention in infants at high risk for celiac disease. *N Engl J Med* 2014; **371:** 1304–15.

29 Liu E, Lee HS, Aronsson CA, et al, for the TEDDY Study Group. Risk of pediatric celiac disease according to HLA haplotype and country. *N Engl J Med* 2014; **371:** 42–49.

30 Andrén Aronsson C, Lee HS, Koletzko S, et al, for the TEDDY Study Group. Effects of gluten intake on risk of celiac disease: a case-control study on a Swedish birth cohort. *Clin Gastroenterol Hepatol* 2016; **14:** 403–09.

31 Crespo-Escobar P, Mearin ML, Hervás D, et al. The role of gluten consumption at an early age in celiac disease development: a further analysis of the prospective PreventCD cohort study. *Am J Clin Nutr* 2017; **105:** 890–96.

32 Szajewska H, Shamir R, Mearin L, et al. Gluten introduction and the risk of coeliac disease: a position paper by the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition. *J Pediatr Gastroenterol Nutr* 2016; **62:** 507–13.

33 Pinto-Sánchez MI, Verdu EF, Liu E, et al. Gluten introduction to infant feeding and risk of celiac disease: systematic review and meta-analysis. *J Pediatr* 2016; **168**: 132–43.

34 Lebwohl B, Green PH, Murray JA, Ludvigsson JF. Season of birth in a nationwide cohort of coeliac disease patients. *Arch Dis Child* 2013; **98:** 48–51.

35 Tanpowpong P, Obuch JC, Jiang H, et al. Multicenter study on season of birth and celiac disease: evidence for a new theoretical model of pathogenesis. *J Pediatr* 2013; **162:** 501–04.

36 Mårild K, Stephansson O, Montgomery S, Murray JA, Ludvigsson JF. Pregnancy outcome and risk of celiac disease in offspring: a nationwide case-control study. *Gastroenterology* 2012; **142:** 39–45.

37 Lionetti E, Castellaneta S, Francavilla R, Pulvirenti A, Catassi C, for the SIGENP Working Group of Weaning and CD Risk. Mode of delivery and risk of celiac disease: risk of celiac disease and age at gluten introduction cohort study. *J Pediatr* 2017; **184**: 81–86.

38 Canova C, Zabeo V, Pitter G, et al. Association of maternal education, early infections, and antibiotic use with celiac disease: a population-based birth cohort study in northeastern Italy. *Am J Epidemiol* 2014; **180:** 76–85.

39 Stene LC, Honeyman MC, Hoffenberg EJ, et al. Rotavirus infection frequency and risk of celiac disease autoimmunity in early childhood: a longitudinal study. *Am J Gastroenterol* 2006; **101:** 2333–40.

40 Riddle MS, Murray JA, Cash BD, Pimentel M, Porter CK. Pathogen-specific risk of celiac disease following bacterial causes of foodborne illness: a retrospective cohort study. *Dig Dis Sci* 2013; **58:** 3242–45.

41 Kemppainen KM, Lynch KF, Liu E, et al, for the TEDDY Study Group. Factors that increase risk of celiac disease autoimmunity after a gastrointestinal infection in early life. *Clin Gastroenterol Hepatol* 2017; **15:** 694–702.

42 Mårild K, Kahrs CR, Tapia G, Stene LC, Størdal K. Infections and risk of celiac disease in childhood: a prospective nationwide cohort study. *Am J Gastroenterol* 2015; **110:** 1475–84.

43 Mårild K, Ye W, Lebwohl B, et al. Antibiotic exposure and the development of coeliac disease: a nationwide case-control study. *BMC Gastroenterol* 2013; **13**: 109.

44 Lebwohl B, Spechler SJ, Wang TC, Green PH, Ludvigsson JF. Use of proton pump inhibitors and subsequent risk of celiac disease. *Dig Liver Dis* 2014; **46:** 36–40.

45 Størdal K, Haugen M, Brantsæter AL, Lundin KE, Stene LC. Association between maternal iron supplementation during pregnancy and risk of celiac disease in children. *Clin Gastroenterol Hepatol* 2014; **12:** 624–31.

46 Mårild K, Ludvigsson J, Sanz Y, Ludvigsson JF. Antibiotic exposure in pregnancy and risk of coeliac disease in offspring: a cohort study. *BMC Gastroenterol* 2014; **14**: 75.

47 Lebwohl B, Blaser MJ, Ludvigsson JF, et al. Decreased risk of celiac disease in patients with *Helicobacter pylori* colonization. *Am J Epidemiol* 2013; **178:** 1721–30.

48 Bouziat R, Hinterleitner R, Brown JJ, et al. Reovirus infection triggers inflammatory responses to dietary antigens and development of celiac disease. *Science* 2017; **356:** 44–50.

49 Galipeau HJ, McCarville JL, Huebener S, et al. Intestinal microbiota modulates gluten-induced immunopathology in humanized mice. *Am J Pathol* 2015; **185:** 2969–82.

50 Nistal E, Caminero A, Vivas S, et al. Differences in faecal bacteria populations and faecal bacteria metabolism in healthy adults and celiac disease patients. *Biochimie* 2012; **94:** 1724–29.

51 Nadal I, Donat E, Ribes-Koninckx C, Calabuig M, Sanz Y. Imbalance in the composition of the duodenal microbiota of children with coeliac disease. *J Med Microbiol* 2007; **56:** 1669–74.

52 Nistal E, Caminero A, Herrán AR, et al. Study of duodenal bacterial communities by 16S rRNA gene analysis in adults with active celiac disease vs non-celiac disease controls. *J Appl Microbiol* 2016; **120:** 1691–700.

53 Nistal E, Caminero A, Herrán AR, et al. Differences of small intestinal bacteria populations in adults and children with/without celiac disease: effect of age, gluten diet, and disease. *Inflamm Bowel Dis* 2012; **18:** 649–56.

54 De Palma G, Nadal I, Collado MC, Sanz Y. Effects of a gluten-free diet on gut microbiota and immune function in healthy adult human subjects. *Br J Nutr* 2009; **102:** 1154–60.

55 Olivares M, Neef A, Castillejo G, et al. The HLA-DQ2 genotype selects for early intestinal microbiota composition in infants at high risk of developing coeliac disease. *Gut* 2015; **64:** 406–17.

56 Rubio-Tapia A, Ludvigsson JF, Brantner TL, Murray JA, Everhart JE. The prevalence of celiac disease in the United States. *Am J Gastroenterol* 2012; **107:** 1538–44.

57 Choung RS, Larson SA, Khaleghi S, et al. Prevalence and morbidity of undiagnosed celiac disease from a community-based study. *Gastroenterology* 2017; **152:** 830–39.

58 Mustalahti K, Catassi C, Reunanen A, et al, for the Coeliac EU Cluster, Project Epidemiology. The prevalence of celiac disease in Europe: results of a centralized, international mass screening project. *Ann Med* 2010; **42:** 587–95.

59 Choung RS, Ditah IC, Nadeau AM, et al. Trends and racial/ethnic disparities in gluten-sensitive problems in the United States: findings from the National Health and Nutrition Examination Surveys from 1988 to 2012. *Am J Gastroenterol* 2015; **110:** 455–61.

60 Almeida RC, Gandolfi L, De Nazaré Klautau-Guimarães M, et al. Does celiac disease occur in Afro-derived Brazilian populations? *Am J Hum Biol* 2012; **24:** 710–12.

61 Ramakrishna BS, Makharia GK, Chetri K, et al. Prevalence of adult celiac disease in India: regional variations and associations. *Am J Gastroenterol* 2016; **111:** 115–23.

62 Krigel A, Turner KO, Makharia GK, Green PH, Genta RM, Lebwohl B. Ethnic variations in duodenal villous atrophy consistent with celiac disease in the United States. *Clin Gastroenterol Hepatol* 2016; **14:** 1105–11.

63 Rubio-Tapia A, Kyle RA, Kaplan EL, et al. Increased prevalence and mortality in undiagnosed celiac disease. *Gastroenterology* 2009; **137:** 88–93.

64 Catassi C, Kryszak D, Bhatti B, et al. Natural history of celiac disease autoimmunity in a USA cohort followed since 1974. *Ann Med* 2010; **42:** 530–38.

65 Lohi S, Mustalahti K, Kaukinen K, et al. Increasing prevalence of coeliac disease over time. *Aliment Pharmacol Ther* 2007; **26:** 1217–25.

66 Myléus A, Ivarsson A, Webb C, et al. Celiac disease revealed in 3% of Swedish 12-year-olds born during an epidemic. *J Pediatr Gastroenterol Nutr* 2009; **49:** 170–76.

67 Choung RS, Unalp-Arida A, Ruhl CE, Brantner TL, Everhart JE, Murray JA. Less hidden celiac disease but increased gluten avoidance without a diagnosis in the United States: findings from the National Health and Nutrition Examination surveys from 2009 to 2014. *Mayo Clin Proc* 2016; published online Dec 5. DOI:10.1016/j.mayocp.2016.10.012.

68 Gasbarrini G, Miele L, Malandrino N, et al. Celiac disease in the 21st century: issues of under- and over-diagnosis. *Int J Immunopathol Pharmacol* 2009; **22:** 1–7.

69 Collin P, Huhtala H, Virta L, Kekkonen L, Reunala T. Diagnosis of celiac disease in clinical practice: physician's alertness to the condition essential. *J Clin Gastroenterol* 2007; **41:** 152–56.

70 Ludvigsson JF, Leffler DA, Bai JC, et al. The Oslo definitions for coeliac disease and related terms. *Gut* 2013; **62:** 43–52.

71 Downey L, Houten R, Murch S, Longson D, for the Guideline Development Group. Recognition, assessment, and management of coeliac disease: summary of updated NICE guidance. *BMJ* 2015; **351:** h4513.

72 Gray AM, Papanicolas IN. Impact of symptoms on quality of life before and after diagnosis of coeliac disease: results from a UK population survey. *BMC Health Serv Res* 2010; **10**: 105.

73 Rubio-Tapia A, Hill ID, Kelly CP, Calderwood AH, Murray JA, for the American College of Gastroenterology. ACG clinical guidelines: diagnosis and management of celiac disease. *Am J Gastroenterol* 2013; **108:** 656–76.

74 Ludvigsson JF, Bai JC, Biagi F, et al, for the BSG Coeliac Disease Guidelines Development Group, British Society of Gastroenterology. Diagnosis and management of adult coeliac disease: guidelines from the British Society of Gastroenterology. *Gut* 2014; **63:** 1210–28.

75 Hopper AD, Hadjivassiliou M, Hurlstone DP, et al. What is the role of serologic testing in celiac disease? A prospective, biopsy-confirmed study with economic analysis. *Clin Gastroenterol Hepatol* 2008; **6:** 314–20.

76 Leffler DA, Schuppan D. Update on serologic testing in celiac disease. *Am J Gastroenterol* 2010; **105:** 2520–24.

77 Lewis NR, Scott BB. Meta-analysis: deamidated gliadin peptide antibody and tissue transglutaminase antibody compared as screening tests for coeliac disease. *Aliment Pharmacol Ther* 2010; **31:** 73–81.

78 Hoerter NA, Shannahan SE, Suarez J, et al. Diagnostic yield of isolated deamidated gliadin peptide antibody elevation for celiac disease. *Dig Dis Sci* 2017; **62:** 1272–76.

79 Giersiepen K, Lelgemann M, Stuhldreher N, et al, for the ESPGHAN Working Group on Coeliac Disease Diagnosis. Accuracy of diagnostic antibody tests for coeliac disease in children: summary of an evidence report. *J Pediatr Gastroenterol Nutr* 2012; **54:** 229–41.

80 Leffler D, Schuppan D, Pallav K, et al. Kinetics of the histological, serological and symptomatic responses to gluten challenge in adults with coeliac disease. *Gut* 2013; **62:** 996–1004.

81 Raju SA, Mooney PD, Aziz I, Kurien M, Sanders DS. Letter: gluten challenge in the era of noncoeliac gluten sensitivity—a change in clinical practice? *Aliment Pharmacol Ther* 2016; **43:** 656.

82 Brocchi E, Corazza GR, Caletti G, Treggiari EA, Barbara L, Gasbarrini G. Endoscopic demonstration of loss of duodenal folds in the diagnosis of celiac disease. *N Engl J Med* 1988; **319:** 741–44.

83 Brocchi E, Tomassetti P, Misitano B, et al. Endoscopic markers in adult coeliac disease. *Dig Liver Dis* 2002; **34:** 177–82.

84 Shah VH, Rotterdam H, Kotler DP, Fasano A, Green PH. All that scallops is not celiac disease. *Gastrointest Endosc* 2000; **51:** 717–20.

85 Biesiekierski JR, Newnham ED, Irving PM, et al. Gluten causes gastrointestinal symptoms in subjects without celiac disease: a double-blind randomized placebo-controlled trial. *Am J Gastroenterol* 2011; **106:** 508–14.

86 Husby S, Koletzko S, Korponay-Szabó IR, et al, for the ESPGHAN Working Group on Coeliac Disease Diagnosis; ESPGHAN Gastroenterology Committee, European Society for Pediatric Gastroenterology, Hepatology, and Nutrition. European Society For Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease. *J Pediatr Gastroenterol Nutr* 2012; **54:** 136–60.

87 Sugai E, Hwang HJ, Vázquez H, et al. Should ESPGHAN guidelines for serologic diagnosis of celiac disease be used in adults? A prospective analysis in an adult patient cohort with high pretest probability. *Am J Gastroenterol* 2015; **110:** 1504–05.

88 Taavela J, Koskinen O, Huhtala H, et al. Validation of morphometric analyses of small-intestinal biopsy readouts in celiac disease. *PLoS One* 2013; **8:** e76163.

89 Mooney PD, Kurien M, Evans KE, et al. Clinical and immunologic features of ultra-short celiac disease. *Gastroenterology* 2016; **150:** 1125–34.

90 Stoven SA, Choung RS, Rubio-Tapia A, et al. Analysis of biopsies from duodenal bulbs of all endoscopy patients increases detection of abnormalities but has a minimal effect on diagnosis of celiac disease. *Clin Gastroenterol Hepatol* 2016; **14:** 1582–88.

91 Latorre M , Lagana SM, Freedberg DE, et al. Endoscopic biopsy technique in the diagnosis of celiac disease: one bite or two? *Gastrointest Endosc* 2015; **81:** 1228–33.

92 Lebwohl B, Kapel RC, Neugut AI, Green PH, Genta RM. Adherence to biopsy guidelines increases celiac disease diagnosis. *Gastrointest Endosc* 2011; **74:** 103–09.

93 Sanders DS, Hurlstone DP, Stokes RO, et al. Changing face of adult coeliac disease: experience of a single university hospital in South Yorkshire. *Postgrad Med J* 2002; **78:** 31–33.

94 Lebwohl B, Bhagat G, Markoff S, et al. Prior endoscopy in patients with newly diagnosed celiac disease: a missed opportunity? *Dig Dis Sci* 2013; **58:** 1293–98.

95 Pallav K, Kabbani T, Tariq S, Vanga R, Kelly CP, Leffler DA. Clinical utility of celiac disease-associated HLA testing. *Dig Dis Sci* 2014; **59:** 2199–206.

96 Chang MS, Rubin M, Lewis SK, Green PH. Diagnosing celiac disease by video capsule endoscopy (VCE) when esophagogastroduodenoscopy (EGD) and biopsy is unable to provide a diagnosis: a case series. *BMC Gastroenterol* 2012; **12**: 90.

97 Murray JA, Rubio-Tapia A, Van Dyke CT, et al. Mucosal atrophy in celiac disease: extent of involvement, correlation with clinical presentation, and response to treatment. *Clin Gastroenterol Hepatol* 2008; **6:** 186–93.

98 Collin P, Kaukinen K, Vogelsang H, et al. Antiendomysial and antihuman recombinant tissue transglutaminase antibodies in the diagnosis of coeliac disease: a biopsy-proven European multicentre study. *Eur J Gastroenterol Hepatol* 2005; **17:** 85–91.

99 Salmi TT, Collin P, Korponay-Szabó IR, et al. Endomysial antibody-negative coeliac disease: clinical characteristics and intestinal autoantibody deposits. *Gut* 2006; **55:** 1746–53.

100 Volta U, Caio G, Boschetti E, et al. Seronegative celiac disease: shedding light on an obscure clinical entity. *Dig Liver Dis* 2016; **48:** 1018–22.

101 Rostami K, Kerckhaert J, Tiemessen R, et al. Sensitivity of antiendomysium and antigliadin antibodies in untreated celiac disease: disappointing in clinical practice. *Am J Gastroenterol* 1999; **94:** 888–94.

102 Aziz I, Peerally MF, Barnes JH, et al. The clinical and phenotypical assessment of seronegative villous atrophy; a prospective UK centre experience evaluating 200 adult cases over a 15-year period (2000–2015). *Gut* 2016; published online Sept 7. DOI:10.1136/gutjnl-2016–312271.

103 DeGaetani M, Tennyson CA, Lebwohl B, et al. Villous atrophy and negative celiac serology: a diagnostic and therapeutic dilemma. *Am J Gastroenterol* 2013; **108:** 647–53.

104 Godfrey JD, Brantner TL, Brinjikji W, et al. Morbidity and mortality among older individuals with undiagnosed celiac disease. *Gastroenterology* 2010; **139:** 763–69.

105 Bakker SF, Tushuizen ME, von Blomberg BM, Bontkes HJ, Mulder CJ, Simsek S. Screening for coeliac disease in adult patients with type 1 diabetes mellitus: myths, facts and controversy. *Diabetol Metab Syndr* 2016; **8**: 51.

106 Mahmud FH, De Melo EN, Noordin K, et al. The Celiac Disease and Diabetes-Dietary Intervention and Evaluation Trial (CD-DIET) protocol: a randomised controlled study to evaluate treatment of asymptomatic coeliac disease in type 1 diabetes. *BMJ Open* 2015; **5:** e008097.

107 Murray JA, Watson T, Clearman B, Mitros F. Effect of a gluten-free diet on gastrointestinal symptoms in celiac disease. *Am J Clin Nutr* 2004; **79:** 669–73.

108 Lee AR, Ng DL, Zivin J, Green PH. Economic burden of a gluten-free diet. *J Hum Nutr Diet* 2007; **20:** 423–30.

109 Shah S, Akbari M, Kelly CP, et al. Celiac disease has higher treatment burden than common medical conditions. *Gastroenterology* 2012; **142:** S87.

110 England CY, Nicholls AM. Advice available on the Internet for people with coeliac disease: an evaluation of the quality of websites. *J Hum Nutr Diet* 2004; **17:** 547–59.

111 Barratt SM, Leeds JS, Sanders DS. Quality of life in Coeliac Disease is determined by perceived degree of difficulty adhering to a gluten-free diet, not the level of dietary adherence ultimately achieved. *J Gastrointestin Liver Dis* 2011; **20:** 241–45.

112 Ludvigsson JF, Agreus L, Ciacci C, et al. Transition from childhood to adulthood in coeliac disease: the Prague consensus report. *Gut* 2016; **65:** 1242–51.

113 Mangione RA, Patel PN, Shin E, Fiebert J. Determining the gluten content of nonprescription drugs: information for patients with celiac disease. *J Am Pharm Assoc* 2011; **51:** 734–37.

114 Zingone F, Abdul Sultan A, Crooks CJ, Tata LJ, Ciacci C, West J. The risk of community-acquired pneumonia among 9803 patients with coeliac disease compared to the general population: a cohort study. *Aliment Pharmacol Ther* 2016; **44:** 57–67.

115 Olmos M, Antelo M, Vazquez H, Smecuol E, Mauriño E, Bai JC. Systematic review and meta-analysis of observational studies on the prevalence of fractures in coeliac disease. *Dig Liver Dis* 2008; **40:** 46–53.

116 Aziz I, Evans KE, Papageorgiou V, Sanders DS. Are patients with coeliac disease seeking alternative therapies to a gluten-free diet? *J Gastrointestin Liver Dis* 2011; **20:** 27–31.

117 McCarville JL, Caminero A, Verdu EF. Pharmacological approaches in celiac disease. *Curr Opin Pharmacol* 2015; **25:** 7–12.

118 Goel G, King T, Daveson AJ, et al. Epitope-specific immunotherapy targeting CD4-positive T cells in coeliac disease: two randomised, double-blind, placebo-controlled phase 1 studies. *Lancet Gastroenterol Hepatol* 2017; published online May 11. DOI:10.1016/S2468-1253(17)30110-3.

119 Leffler DA, Kelly CP, Green PH, et al. Larazotide acetate for persistent symptoms of celiac disease despite a gluten-free diet: a randomized controlled trial. *Gastroenterology* 2015; **148:** 1311–19.

120 Murray JA, Kelly CP, Green PH, et al, for the CeliAction Study Group of Investigators. No difference between latiglutenase and placebo in reducing villous atrophy or improving symptoms in patients with symptomatic celiac disease. *Gastroenterology* 2017; **152:** 787–98.

121 Leffler DA, Dennis M, Hyett B, Kelly E, Schuppan D, Kelly CP. Etiologies and predictors of diagnosis in nonresponsive celiac disease. *Clin Gastroenterol Hepatol* 2007; **5:** 445–50.

122 Rubio-Tapia A, Barton SH, Murray JA. Celiac disease and persistent symptoms. *Clin Gastroenterol Hepatol* 2011; **9:** 13–17.

123 Lebwohl B, Murray JA, Rubio-Tapia A, Green PH, Ludvigsson JF. Predictors of persistent villous atrophy in coeliac disease: a population-based study. *Aliment Pharmacol Ther* 2014; **39:** 488–95.

124 Leonard MM, Weir DC, DeGroote M, et al. Value of IgA tTG in predicting mucosal recovery in children with celiac disease on a gluten-free diet. *J Pediatr Gastroenterol Nutr* 2017; **64:** 286–91.

125 Lebwohl B, Granath F, Ekbom A, et al. Mucosal healing and risk of lymphoproliferative malignancy in celiac disease. *Ann Intern Med* 2013; **159:** 169–75.

126 Lebwohl B, Michaëlsson K, Green PH, Ludvigsson JF. Persistent mucosal damage and risk of fracture in celiac disease. *J Clin Endocrinol Metab* 2014; **99:** 609–16.

127 Sharkey LM, Corbett G, Currie E, Lee J, Sweeney N, Woodward JM. Optimising delivery of care in coeliac disease—comparison of the benefits of repeat biopsy and serological follow-up. *Aliment Pharmacol Ther* 2013; **38:** 1278–91.

128 Hollon JR, Cureton PA, Martin ML, Puppa EL, Fasano A. Trace gluten contamination may play a role in mucosal and clinical recovery in a subgroup of diet-adherent non-responsive celiac disease patients. *BMC Gastroenterol* 2013; **13**: 40.

129 Cellier C, et al, for the French Coeliac Disease Study Group. Refractory sprue, coeliac disease, and enteropathy-associated T-cell lymphoma. *Lancet* 2000; **356:** 203–08.

130 Rubio-Tapia A, Kelly DG, Lahr BD, Dogan A, Wu TT, Murray JA. Clinical staging and survival in refractory celiac disease: a single center experience. *Gastroenterology* 2009; **136:** 99–107.

131 Rubio-Tapia A, Malamut G, Verbeek WH, et al. Creation of a model to predict survival in patients with refractory coeliac disease using a multinational registry. *Aliment Pharmacol Ther* 2016; **44:** 704–14.

132 Elfstrom, P, Granath F, Ekström Smedby K, et al. Risk of lymphoproliferative malignancy in relation to small intestinal histopathology among patients with celiac disease. *J Natl Cancer Inst* 2011; **103:** 436–44.

133 Ilus T, Kaukinen K, Virta LJ, Pukkala E, Collin P. Incidence of malignancies in diagnosed celiac patients: a population-based estimate. *Am J Gastroenterol* 2014; **109:** 1471–77.

134 Elfström P, Granath F, Ye W, Ludvigsson JF. Low risk of gastrointestinal cancer among patients with celiac disease, inflammation, or latent celiac disease. *Clin Gastroenterol Hepatol* 2012; **10:** 30–36.

135 Lebwohl B, Stavsky E, Neugut AI, Green PH. Risk of colorectal adenomas in patients with coeliac disease. *Aliment Pharmacol Ther* 2010; **32:** 1037–43.

136 Green PH, Fleischauer AT, Bhagat G, Goyal R, Jabri B, Neugut AI. Risk of malignancy in patients with celiac disease. *Am J Med* 2003; **115:** 191–95.

137 West J, Logan RF, Smith CJ, Hubbard RB, Card TR. Malignancy and mortality in people with coeliac disease: population based cohort study. *BMJ* 2004; **329:** 716–19.

138 Tio M, Cox MR, Eslick GD. Meta-analysis: coeliac disease and the risk of all-cause mortality, any malignancy and lymphoid malignancy. *Aliment Pharmacol Ther* 2012; **35:** 540–51.

139 Logan RF, Rifkind EA, Turner ID, Ferguson A. Mortality in celiac disease. *Gastroenterology* 1989; **97:** 265–71.

140 Ludvigsson JF, Montgomery SM, Ekbom A, Brandt L, Granath F. Small-intestinal histopathology and mortality risk in celiac disease. *JAMA* 2009; **302:** 1171–78.

141 Johnston SD, Watson RG, McMillan SA, Sloan J, Love AH. Coeliac disease detected by screening is not silent—simply unrecognized. *QJM* 1998; **91:** 853–60.

142 Lohi S, Mäki M, Rissanen H, Knekt P, Reunanen A, Kaukinen K. Prognosis of unrecognized coeliac disease as regards mortality: a population-based cohort study. *Ann Med* 2009; **41:** 508–15.

143 Moreno ML, Rodríguez-Herrera A, Sousa C, Comino I. Biomarkers to monitor gluten-free diet compliance in celiac patients. *Nutrients* 2017; **9**.

144 Comino I, Fernández-Bañares F, Esteve M, et al. Fecal gluten peptides reveal limitations of serological tests and food questionnaires for monitoring gluten-free diet in celiac disease patients. *Am J Gastroenterol* 2016; **111:** 1456–65.