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REVIEW

Celiac disease: Prevalence, diagnosis, pathogenesis and treatment

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Abstract

Celiac disease (CD) is one of the most common diseases, resulting from both environmental (gluten) and genetic factors [human leukocyte antigen (HLA) and non-HLA genes]. The prevalence of CD has been estimated to approximate 0.5%-1% in different parts of the world. However, the population with diabetes, autoimmune disorder or relatives of CD individuals have even higher risk for the development of CD, at least in part, because of shared HLA typing. Gliadin gains access to the basal surface of the epithelium, and interact directly with the immune system, *via* both trans- and para-cellular routes. From a diagnostic perspective, symptoms may be viewed as either "typical" or "atypical". In both positive serological screening results suggestive of CD, should lead to small bowel biopsy followed by a favourable clinical and serological response to the gluten-free diet (GFD) to confirm the diagnosis. Positive anti-tissue transglutaminase antibody or anti-endomysial antibody during the clinical course helps to confirm the diagnosis of CD because of their over 99% specificities when small bowel villous atrophy is present on biopsy. Currently, the only treatment available for CD individuals is a strict life-long GFD. A greater understanding of the pathogenesis of CD allows alternative future CD treatments to hydrolyse toxic gliadin peptide, prevent toxic gliadin peptide absorption, blockage of selective deamidation of specific glutamine residues by tissue, restore immune tolerance towards gluten, modulation of immune response to dietary gliadin, and restoration of intestinal architecture.

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Key words: Celiac disease; Demography; Diagnosis; Pathogenesis; Treatment

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INTRODUCTION

Celiac disease (CD) is a life-long gluten-sensitive autoimmune disease of the small intestine affecting genetically susceptible individuals worldwide. CD individuals may present gastrointestinal symptoms, extraintestinal symptoms or no signs of symptoms. The classical symptoms include gastrointestinal-related symptoms such as diarrhea, steatorrhea and weight loss due to malabsorption. About 50% of CD patients present extraintestinal or

atypical symptoms, such as anemia, osteoporosis, dermatitis herpetiformis, neurological problems and dental enamel hypoplasia[1-3]. The variable clinical picture of CD is due to having both genetical and immunological bases, with age of onset, extent of mucosal injury, dietary habits, and gender[4], affecting the clinical manifestation of the disease.

CD diagnosis is based on presence of predisposing genetic factor human leukocyte antigen (HLA) DQ2/8, with positive biopsy and serological antibodies upon gluten contained diet. The spectrum of CD may present in different forms[5]. The classical form may be diagnosed at any age of life and is often characterized by crypt hyperplasia and villous atrophy along with features of malabsorption. The atypical form is characterized by positive celiac serology, limited abnormalities of the small intestinal mucosa or no intestinal symptoms, but associated extraintestinal conditions such as osteoporosis, peripheral neuropathy, anemia and infertility. The latent form is defined by presence of predisposing gene HLA-DQ2 and/or HLA-DQ8, normal intestinal mucosa and, possible positive serology. Extraintestinal features and biopsies of the small bowel show alterations with gluten intake (i.e., gluten-sensitive). Rarely, usually after age 50 years[6], some that have initially responded to a gluten-free diet (GFD), develop recurrent symptoms and biopsy changes despite a GFD. This is the refractory form[7]. If no response to GFD was initially documented, however, the use of sprue-like intestinal disease or unclassified sprue has been used.

PREVALENCE

CD originally thought to almost exclusively affect white Europeans, is now known to be widely distributed worldwide[8]. Epidemiological studies conducted in areas supposedly free of CD, including Africa, the Middle East, Asia, and South America, show that the disease was previously underdiagnosed[9]. This provides evidence that CD is one of the most common genetic diseases, resulting from both environmental (gluten) and genetic (HLA and non-HLA genes) factors.

The world distribution of CD seems to have followed the mankind wheat consumption and the migratory flows. Man originally fed on meat, fruit and vegetables, with no exposer to gluten-containing cereals. It was only about 10 000 years ago in a small region called the "Fertile Crescent" of the Middle-East (including Anatolia (Southern Turkey), Lebanon, Syria, Palestine and Iraq) where wild wheat and barley grains successfully cultivated due to favorable environmental conditions. In the Fertile Crescent some tribes changed from nomadic to stable settlement style of living because land cultivation permitted food storage, and later migrated westwards to obtain new lands for cultivation. These persons spread through the Mediterranean area (Northern Africa, Southern Europe) and Central Europe. The expansion continued from 9000 to 4000 BC by which time the cultivation of wheat and barley had spread all over the Old Continent, also reaching Northern Europe (Ireland, Denmark and the Scandinavian countries). This expansion in farming was due to the diffusion of agricultural practices and replacement of local inhabitants by descendants from the Middle-East[10]. Hence, the European and North-African populations share genetic background with the peoples of Middle-East origin.

In the last few years a number of studies in different populations have been carried out using molecular genetics methods to identify genes causing CD. CD-predisposing genetic loci are *CELIAC1* on chromosome 6, *CELIAC2* on chromosome 5q31-33[11], *CELIAC3* on chromosome 2q33[12], and *CELIAC4* on chromosome 19p13.1[13]. *PARD3* and *MAGI2* tight junction genes associations have also been reported in Dutch CD or ulcerative colitis patients, suggesting a common intestinal defect in these two conditions[14]. Another gene expressed in major histocompatibility complex (MHC) I antigen presenting cell is *HLA B8*, was found to be associated with CD in Algeria[15], Iraq[16,17] and Turkey[18]. Moreover, atypical CD Saharawi patients were found to over-express the MHC class I chain-related gene A (MICA) allele 5.1[15], which have also been reported in Western countries[19]. Increased prevalence of HLA-A25 in Turkish children with CD was also reported, suggesting that this genotype is particularly encountered among this population[18], with no association described in Western countries.

Useful Background: Genes causing CD CELIAC1 on chromosome 6 (*HLA-DQ2* and *HLA-DQ8*); CELIAC2 on chromosome 5q31-33[11]; CELIAC3 on chromosome 2q33 (containing T lymphocyte regulatory genes *CD28*, *CTLA4* and *ICOS*)[12]; and CELIAC4 (myosin IXB gene, *MYO9XB*) on chromosome 19p13.1[13].

HLA genotype contributes to the genetic risk for CD at 30%-50%[20,21]. Non-HLA genes contribute more evidence to the CD genetic background than the HLA genes, but each by itself contributes only a modest to the disease development. Hence, it is reasonable to assume that the susceptibility to CD involves with polymorphic genes that influence the immune response to gluten, as shown for the HLA-linked genes[22].

Ninety percent of European patients with CD carry the HLA-DQ2 molecule, encoded either in cis on the HLA-DQA1*0501-DQB1*0201 haplotype (HLA-DQ2.5cis) or in trans on the HLA-DQA1*0505 DQB1*0301/DQA1*0201-DQB1*0202 haplotypes (HLA-DQ2.5trans). Approximately 5% express HLA-DQ8, encoded by HLA-DQA1*0301-DQB1*0302. The majority of the remainder carry the HLA-DQA1*0201-DQB1*0202 haplotype[20]. With genetic testing, DQ2 is almost synonymous with DQB1*02, a gene with two common alleles designated DQB1*0201 and DQB1*0202. The DQ2 frequency in Caucasian in Western Europe populations has been estimated at 20%-30%, and relatively high frequencies also occur in Northern and Western Africa, the Middle East and central Asia[23]. Thereafter, the overall frequency of DQ2 declines from West to East with low frequencies in

Table 1 Frequency of human leukocyte antigen-DQ2, encoded by human leukocyte antigen-DQB1*02 and human leukocyte antigen-DQ8, encoded by human leukocyte antigen-DQA1*0301-DQB1*0302

< 5%	5%-20%	20%
HLA-DQ2		
Albania	Belarus Algeria	Algeria
Canada BC (Athabaskans)	Cameroon	Australia
Cook Islands	Congo	Belgium
Indonesia	Costa Rica	Central African Republic
Japan	China	Croatia
Jordan	Cuba	England
Papua New Guinea	Ecuador Africans	Equatorial Guinea Bioko Island
Philippines	France	Ethiopia
Samoa	India	Germany
	Malaysia	Greece
	Mexico	Iran
	Poland	Ireland South
	Russia	Israel
	Singapore	Italy
	South Korea	Mongolia
	Spain	New Zealand
	Sri Lanka	Pakistan
	Sweden	Saudi Arabia
	Taiwan, China	Slovenia
	Thailand	Tunesia
	Turkey	United States
	Uganda	
	Ukraine	
	Vietnam	
HLA-DQ8		
Australia	Algeria	Argentina
China	Belgium	Ecuador
Georgia	Brazil	Ethiopia
Greece	Canada BC (Athabaskans)	Mexico
North India	Croatia	Venezuela
Spain	England Caucasoid	
Uganda	France	
	South India	
	Israel	
	Italy	
	Japan	
	Russia	
	South Korea	
	Tunisia	
	Turkey	
	Ukraine	
	United States	
	European American	

Estimates are based on studies included in a comprehensive Internet website[24]. In several countries, the frequency is not known. HLA: Human leukocyte antigen.

populations in South-East Asia and the virtual absence of DQ2 in Japan (Table 1). DQ8 frequency has a worldwide distribution, whereas DQ2.5, is common in South and Central America; approximately 90% of Amerindian populations carry DQ8 and may display the celiac phenotype[24,25]. The frequency of DQ8 population is shown in Table 1.

In the past, the prevalence of CD had been underestimated, but it is now regarded one of the most common genetic disorders in the West with 1% prevalence[26-28]. In-

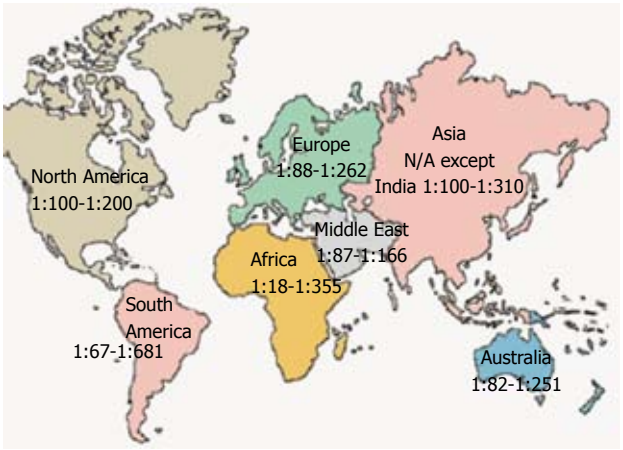

Figure 1 Prevalence of celiac disease worldwide. N/A: Not available.

terestingly, there is increased prevalence of CD amongst women compared to men with male:female ratio of 1:2.8[29]. This could be due to the finding that men with CD were diagnosed at an older age[30]. Indeed, there have been reported CD cases among immigrant children native of Eastern Europe, Northern, West and East Africa, the Middle East and Southern Asia, according to their acquisition of Western dietary practices (i.e., short period or lack of breast feeding and early weaning with a great amount of gluten intake)[31]. This suggests that many persons may have the genetic predisposition to CD but the clinical presentation only occurs when there is sufficient gluten in the diet.

Normal at-risk persons

In several parts of the world, the presence of the combination of antibody (serum tissue transglutaminase and endomysial autoantibodies) positivity and an HLA haplotype associated with CD is predictive of small-bowel abnormalities indicative of CD. For the majority of countries, the CD prevalence is unknown. Figure 1 shows a range of estimated normal at-risk CD prevalence in continents and nations around the globe. It must be noted that some studies report prevalence of CD based on serology, others on celiac compatible small bowel biopsies and a few on serology, biopsy and response to gluten challenge.

North America

CD prevalence in North American and Europe were found to be similar in symptomatic patients and not-at-risk subjects. In the United States, CD is believed to affect 0.5%-1.0% of the general population[32]. The study by Fasano *et al*[28] on serum antibody and biopsy screening were performed for a total of 13 145 United States subjects: first-degree (n = 4508) and second-degree relatives of patients (n = 1275) with biopsy-proven CD, symptomatic patients (n = 3236) (with either gastrointestinal symptoms or a disorder associated with CD), and not-at-risk individuals (n = 4126). The overall CD prevalence is 1:133 in the not-at-risk groups, whereas in the at-

Table 2 Prevalence of celiac disease in Europeans based on unselected population serological screenings[36-38] (adapted)

Countries	Prevalence
Czechoslovakia	0.193
Estonia	0.103
Finland	0.110
Hungary	0.101
Ireland	0.126
Italy	0.115
Norway	0.224
Portugal	0.135
Spain	0.124
Sweden	0.174
Switzerland	0.133
Netherlands	0.179
United Kingdom	0.111

risk group, the prevalence is 1:22 in first-degree relatives, 1:39 in second-degree relatives, and 1:56 in symptomatic patients[28].

South America

In South America, CD had been historically considered a rare disorder and the prevalence investigations have not been extensively studied. However, during the last few years studies in Brazil disclosed a prevalence of 1:681 in healthy blood donors[33] and 1:473 among adult outpatients attending a clinical laboratory for routine blood testing[34]. In an urban area of Argentina, the overall prevalence of CD, among 2000 adults from the general population (996 women; median age 29 year, range 16-79 year) was 1:167, with prevalence in women double that for men[35]. The high CD prevalence in Argentina could be correlated with HLA DQ8 (> 20%) in the Argentina population.

Europe

The overall prevalence of CD in Western populations is close to 1% (1:100) and may be higher in Northern European countries (Table 2)[36-38]. The Scandinavian countries, Ireland, and the United Kingdom population tended to show a higher prevalence of CD of approximately 1.0%-1.5%, although there also were studies that showed a lower prevalence in these countries. A study of small-intestinal biopsy obtained from healthy Dutch blood donors at Arnhem and Nijmegen Blood Donation Centers shows that the prevalence of CD-compatible biopsies of 1:330[39]. The prevalence of CD among 3654 children (age range, 7-16 years) in Finland was at least 1:99 based on serum autoantibodies and small-bowel abnormalities[40]. The prevalence of CD in Northern Spain in the general population was 1:389[41], 1:132 (0.75%) in Eastern Switzerland adolescents[42].

Africa

In Northern African populations (including Morocco, Algeria, Tunisia, Libya and Egypt) higher incidences of 0.28%-5.6% of CD have recently been reported in the general population[43-46]. The prevalence of CD in asymptomatic Tunisian school children was estimated to be about 1:157, which is close to the European prevalence. In this respect, the highest world frequency of 16.4% is reported in the CD associated with Insulin Dependent Diabetes Mellitus[47], in Oran (Algeria). A recent serological screening in 2500 Tunisian healthy blood donors[45], showed similar that the prevalence of anti-endomysium antibodies in the general population of 1:355, to that of Europeans. Due to high wheat and barley consumption in the North American countries[45], as well as high frequency of CD predisposing DR3-DQ2 haplotypes in these populations[48-50], these high CD frequencies are not surprising.

Saharawi population in North Africa, who are of Arabian and Berberian origin, having a high degree of cognation and live as refugees in the Sahara desert (Algeria), has the highest prevalence of CD (5.6%) known in the world today[43,49]. This elevated prevalence in this population may be explained both by genetic factors: very high frequency of the DR3-DQ2 haplotype, and by environmental factors: changed of dietary habits in the last few decades. The reduced rates and duration of breast-feeding and increased consumption of gluten in early life as part of the staple diet, supplied by Western countries as humanitarian aids[51], may have played a role in this elevated CD prevalence. However, there are other unknown genetic and environmental factors that explain such a high frequency of CD in the Saharawi people, because there is a much lower prevalence of CD in Sardinia population with similar staple diet consumption and frequencies of DR3-DQ2[52].

Australia and New Zealand

Australia and New Zealand are the two countries having the highest proportion of individuals from Caucasian background, with high prevalence of HLA DQ2 and per capita wheat consumption of > 150 and 75-150 kg per person per year, respectively[23]. Only two prevalence studies have been carried out in these two countries. From a random population of 1064 adults in Christchurch, New Zealand (96% Caucasian), CD was confirmed histologically in all patients with positive serology giving an overall prevalence of 1:82 (1.2%)[53]. A larger study in 3011 adults from a large Caucasian community in Western Australia, revealed an overall prevalence of CD of 1:251 (0.4%) of the population[54].

Asia

CD is likely to be rare in Indonesia, South Korea, Philippines and many smaller Pacific islands because of their low wheat consumption and a low frequency of HLA-DQB1*02. In South-East Asia, HLA-DQB1*02 is often present in more than 5% of the population but CD is predicted to be rare, as staple diets are based on rice. In contrast, prevalence rates that are similar to those in Europe are likely to apply from Pakistan in the South to Kazakhstan in the North. Ancient migration patterns

Table 3 High risk populations for celiac disease[73] (adapted)

Relatives, especially first-degree
Anemia, especially iron deficiency
Osteopenic bone disease
Insulin-dependent diabetes (type 1), especially children
Liver disorders, especially Autoimmune hepatitis and primary biliary cirrhosis
Genetic disorders, including down and Turner's syndrome
Autoimmune endocrinopathy, especially thyroid disease
Skin disorders, particularly dermatitis herpetiformis
Neurological disorders, including ataxia, seizures, myasthenia gravis
Others, including immunoglobulin A nephropathy

that determine the frequency of DQB1*02 would also predict more patients with CD in Western China than in Eastern China[23]. Interestingly, there is one report of CD in three adult descendants of Chinese and Japanese families who migrated to Canada[55].

In genetic studies of CD in India, the appearance of HLA associations is similar to those in Western countries with a frequency of HLA-DQB1*02 of close to 100%[56]. This association is more frequent in the population of Northern and North-Eastern India (16%-27%)[57], than in groups of adults in the Southern state of Tamil Nadu (9%-14%)[58]. The prevalence of CD in India is nearly the same as that in Western Caucasian populations[59]. In Punjab (North-west India) school children, CD frequency was estimated to be 0.3%[60]. This prevalence is probably an underestimation. A retrospective analysis of confirmed cases of CD between 1995 and 2000 in Dayanand Medical College and Hospital (Ludhiana, Punjab) from a total of 202 cases showed an initial of 10 positive cases with a significant growth rate of 79.43% annually with a trend equation increase of 15.49 cases/year[61]. These studies showed that CD is relatively common in Northern India where there has been a history of wheat cultivation from before 1000 BC[62]. Hence, the relative rarity of CD in Southern India reflects the effect of both genetic and environmental factors.

The prevalence in first-degree relatives of North Indian children with CD diagnosed as per the European Society for Pediatric Gastroenterology and Nutrition criteria is 4.4% of the first-degree relatives (85% positive for HLA DQ2/DQ8), which is 14 times higher than that of the general population[63]. There have been reports on clinical experience of biopsy-defined CD in 10 North Indian Immigrants or descendants born in Canada out of 14 Asians diagnosed since 1988 in a single Canadian teaching hospital[55]. Several studies, particularly from Northern and North-West India, have also documented the presence of CD in children presenting with chronic diarrhea[64].

Middle East

It seems likely that the prevalence of CD in the Middle East is similar to that of Europe[65]. CD is a relatively common cause of chronic diarrhea in Iran, Iraq and Kuwait and has been diagnosed in 2%-8% of patients with type 1 diabetes in Iran, Israel and Saudi Arabia[39]. Many of these countries have a per capita wheat consumption that ranks among the highest in the world (> 150 kg per person per year)[23]. Although only a limited number of genetic studies have been carried out, the population of countries such as Iran, Israel, Saudi Arabia and Turkey have a high frequency of HLA-DQB1*02. The prevalence of CD in adult blood donors in Iran, Israel, Syria, Turkey and Anatolia are 1:166[66], 1:157[67], 1:62[68], 1:87[69], 1:100[70], respectively. Similar prevalence rates were determined in surveys of Iranian children (1:165, 0.6%)[71], and Turkish children (1:115, 0.9%)[72].

The prevalence of CD is approximately 0.5%-1% in all parts of the world, except for populations with very low and very high intake of gluten in their diet.

High at-risk persons

In the general celiac population (without classical CD symptoms, e.g., diarrhea or weight loss), there are high risk groups that may have higher CD prevalence rates (Table 3). Among factors that denote a higher risk for CD, the most important factor is familial history of biopsy proven CD with an estimate of 20% or more of first-degree relatives having CD[73]. Some authors observed a higher prevalence in CD siblings as compared to parents[74-76]. A study in Swedish youth (< 20 years old) diagnosed with type 1 diabetes (T1D) confirmed the low prevalence (0.7%) of diagnosed symptomatic CD at initial onset of clinical diagnosis, but document by screening an increasing prevalence of silent CD during a 5-year follow-up to reached an overall prevalence of 10%[77]. Thus, the prevalence of an association with CD in high risk groups may increase over time.

The overall prevalence of CD is highly dependent on the HLA DQ2/DQ8 typing and gluten consumption. The population with positive HLA typing for celiac have high chances of developing celiac symptoms when on high gluten consumption. However, the population with diabetes, autoimmune disorder or relatives of CD individuals) have even higher risk for the development of CD, since they share the same HLA typing.

PATHOGENESIS

CD is an intestinal enteropathy triggered by the ingestion of gliadin and of other related prolamins in genetically predisposed individuals[22,78]. Wheat and related species such as barley and rye also induce CD[79]. A small minority of CD patients also react to oat[80]. Gliadin peptides exert damaging effects since they are resistant to gastrointestinal enzymes[81], they have amino acid sequences that are specific for HLA-DQ2, which is a class Ⅱ major histocompatibility complex, they also have preferred glutamine residues for tissue transglutaminase (tTG)-mediated deamidation[82], and lastly, they affect intestinal permeability[83]. Hence the pathogenesis of CD is dependent on genetic and environmental factors. The environmental factor is mainly ingestion of gluten, while several genes contribute to the genetic predisposition[13]. CD commonly

Table 4 Possible clinical manifestations of celiac disease[8] (printed with permission)

Typical symptoms	Atypical symptoms	Associated conditions
Chronic diarrhea	Secondary to malabsorption	Possible gluten dependent
Failure to thrive	Sideropenic anemia	IDDM
Abdominal distention	Short stature	Autoimmue thyroiditis
	Osteopenia	Autoimmune hepatitis
	Recurrent abortions	Sjogren 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	
	Vasculitis	
	Dilatative cardiomyopathy	
	Hypo/hyperthyroidism	

IgA: Immunoglobulin A; IDDM: Insulin dependent diabetes mellitus.

appears in early childhood, with severe symptoms including chronic diarrhea, abdominal distension, and failure to thrive. In many patients, symptoms may not develop until later in life, when the disease symptoms include fatigue, diarrhea, and weight loss due to malabsorption, anemia, and neurological symptoms (Table 4). Celiac disease is a life-long disorder, and if untreated, it is associated with increased morbidity and mortality[84, 85].

Possible triggers

Genetic predisposition association (HLA, MYO9B), exogenous trigger (gluten), pro-autoimmune genetic background, viral infections, tissue damage, early termination of breastfeeding and gender contribute to the development of CD (Table 5)[86].

Apart from introduction of gluten during the first year of life, infectious agents may play a role in development of CD. Several studies have implicated infections with Adenovirus type 12[87-89], hepatitis C virus[90,91], *Campylobacter jejuni*[92], *Giardia lamblia*[93], Rotavirus[94] and Enterovirus infection[95] as triggers for the development of CD. The immunologic response in persons genetically susceptible

Table 5 The most important factors contributing to the development of celiac disease[86] (printed with permission)

Factors contributing to the onset of celiac disease	Mechanism
Gluten	Elicit T cell responses Induces cytokine production and intestinal lesion
Age of introduction of gluten	Weak gut immune during early childhood
HLA-DQ2 or HLA-DQ8	Gluten presentation
MYO9Bo	Increased permeability of the intestine
Pro-autoimmune genetic background	Shift in Th1/Th2 balance towards Th1
Viral infections	Defect in generation of active tolerance (e.g., regulatory T cells) IFN production
Tissue damage	Tissue damage Increased level of tTG Danger signals
Early termination of breastfeeding	Decreased protection against infections
Gender	Hormone-related pro-autoimmune status

Th1: T helper 1; Th2: T helper 2; tTG: Tissue transglutaminase; HLA: Human leukocyte antigen; IFN: Interferon.

CD may be triggered due shared viral sequence of 8 to 12 amino acids with the toxic gliadin fraction[89]. Other factors such as timing of gluten ingestion and breast feeding cessation may involve in the pathogenesis and disease development of CD[96]. Some initiating factors, such as gluten overload, gastric surgery "unmasking", giving up smoking, and infections can also trigger the disease, which can become apparent in an abrupt manner[97,98].

Prolamin trigger

Gluten is a protein that appears in wheat, barley, rye and oat, compositing of prolamin and glutelin. The majority of the proteins in food responsible for the immune reaction in CD are the prolamins. Prolamins is found in several grains, such as wheat (gliadin), barley (hordein) and rye (secalin), corn (zein) and as a minor protein, avenin in oats. Because of their high glutamine content and specific sequence patterns, prolamins are resistant to gastrointestinal proteolytic enzymes and are excellent substrates for deamidation by tissue transglutaminase.

The incomplete gastrointestinal digestion of gluten leads to the appearance of gluten-derived gliadin peptides such as 33mer (LQLQPFPQPQLPYPQPLPYPQPQL PYPQPQPF) with a variety of characteristics[81]. It contains overlapping T-cell epitopes, and its deamidated form is a potent T-cell stimulator, generating the glutamic acid residues essential for binding to HLA-DQ2 in celiac patients[99]. The ingestion of prolamins from wheat, barley, rye and possibly oats causes histological changes in the small intestine mucosa of celiac patients, leading to a malabsorption syndrome[100]. Clinical symptoms of an autoimmune attack after ingestion of the gluten containing food include digestive symptoms and skin reactions.

Gliadin peptides cause stimulation of the innate and adaptive immune system[83,101,102]. The prototype of pep-

tides effective on innate response is peptide 31-43/49, which has been proved both *in vitro* and *in vivo* to be toxic for CD patients[103,104]. Peptide 31-43 (p31-43) stimulates the synthesis and release of interleukin (IL)-15, a proinflammatory cytokine, that promotes the adaptive immune response[101], involving CD4+ T cells that recognize several deamidated gliadin peptides[82]. Unlike p31-43 which is not immunogenic for T cells, peptide 57-68 (p57-68), which binds to HLA-DQ2/8 molecules, is one of the dominant epitopes recognized by T cells isolated from the intestine of CD patients[82]. The so-called toxic peptides, of which p31-43 is probably the most fully studied, modulate the small-intestinal mucosal biology *via* an innate immune mechanism.

Time of trigger

Several studies related the rise in childhood CD to infant feeding practices[96,105]. Consumption of wheat, barley, and rye in the first 3 mo children have significantly increased the risk of developing CD-associated autoantibodies, as compared with exposure during first 4 to 6 mo[105,106].

Although CD can be diagnosed at any time of life, it is present mostly in either early childhood (between 9 and 24 mo) or in the third or fourth decade of life[8,85,107,108]. In contrast to the 1/1 sex ratio in children, in adulthood it is diagnosed twice in females. Interestingly, celiac disease is also becoming more frequently recognized in the elderly, and in this population, a 1/1 sex ratio has also been noted[109]. Although the "classical" gastrointestinal malabsorption syndrome characterized by diarrhea, steatorrhoea, weight loss, fatigue, and anemia may occur in severe cases, most patients have a milder symptoms such as abdominal discomfort, bloating, indigestion, or non-gastrointestinal symptoms (or no symptoms at all)[8,85,107,108]. Mäki *et al*[2] reported a shift of 5-6 years of age at diagnosis in Finland, with less than 50% of new cases presenting typical gastrointestinal symptoms. In England[110], Scotland[111], Canada[112], and the United States[28], reports have also shown that almost 50% of newly diagnosed CD patients do not present with gastrointestinal symptoms.

GENETICS

Genetics play a strong role in CD, indicated by the high disease concordance in monozygotic twin[113]. The CD prevalence rose to 17.6% in sisters, 10.8% in brothers, and 3.4% in parents[21]. CD is associated with HLA alleles as well as more than 250 other MHC and non-MHC genes. The main genetic factor is the given HLA-DQ genes, i.e., the genes encoding DQ2 or DQ8 in the HLA complex on 6p21. Approximately 95% of celiac have a DQ2 comprised of DQB1*302 and DQA1*03. A small number of individuals lacking either of those heterodimers have DQB1*02 and DQA1*05 alone[20,114]. Gene dosage also affect CD susceptibility; individuals with the heterodimer comprised of DQB1*02 and DQA1*05 and most of the remaining 5% have a DQ8 heterodimer. Homozygous individuals who carry DQB1*02 and DQA1*05 in cis on both chromosomes have a great risk developing complicated forms of CD[115]. A significant higher risk for CD of 1:7 for DQ2 and DQ8 individuals and 1:2518 for subjects lacking all predisposing factors have been determined[21].

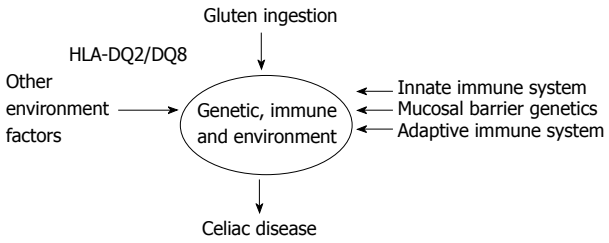

Figure 2 Factors necessary for celiac disease development[96] (adapted). HLA: Human leukocyte antigen.

It is found that 30% of the Caucasian populations carry HLA-DQ2 and most will eat wheat, while only 1 in 100 will develop disease[116]. The remaining susceptibility is thought to be due to a combination of genetic and environmental factors (Figure 2).

HLA-DQ2.5 carriage is necessary for disease development, but it is not sufficient by itself. A combination of other genetic factors influencing the mucosal barrier, the adaptive and the innate immune system also impact the likelihood of disease development. Wheat ingestion is a known environment factor that is necessary for disease development but on top of this, a number of factors such as timing of gluten ingestion and breast feeding cessation may influence disease development[96].

Studies using twins, which are assumed to share environmental factors, have estimated the percentage of non-HLA genetic variants which predispose to disease as approximately 60%[117]. To date a large list of variants have been suggested to predispose to CD through a combination of linkage and association studies, a large number of variants, however, do not stand up to further scrutiny. Only those that have been validated with convincing evidence in multiple populations are mentioned here.

In CD, like many common diseases, this genome wide linkage approach has been fairly unsuccessful at locating variants. Linkage was found to various regions including 5q and 19p, however, the only genomic region that was replicated with some reliability in other populations was 2q33, a region that contains the *CTLA4*[118], *ICOS* and *CD28* genes[117]. *CTLA4* is an excellent candidate gene for involvement in CD not only due to its integral involvement in the suppression of immune responses but also because it has been implicated as a genetic variant that increases susceptibility to T1D.

The prevalence of CD in patients diagnosed with T1D has been estimated at up to 15% in children and 6% in adults[119]. The reason for this association has never been fully elucidated, but common mechanisms within the pathogenesis and genetics of the two conditions may provide some insights. IL-21 region displays CD associations to T1D[120], rheumatoid arthritis[121], Grave's

disease[122] and psoriatic arthritis[123]; but genetic involvement in all these conditions is not currently understood. There is possibility of shared genetic susceptibility to autoimmunity through *IL-2*, *IL-21* locus, both inside and outside of the HLA region, with almost no function identified thus far[116]. Like the studies associated with the HLA-DQ2.5 variant, further identification of the causal variant and its function will provide a unique insight into CD and other autoimmune disease biology.

Immune function

CD is an autoimmune disease associated with the genetic predisposition HLA and tTG autoantigen. tTG is a calcium dependent enzyme that plays a crucial role in CD pathogenesis[124]. tTG mediates ordered and specific deamidation of gliadins, creating an epitope that binds efficiently to DQ2 and is recognized by gut-derived T cells[125]. During gluten consumption, these tTG autoantibodies are produced by the mucosa of the small intestine, and detected in patients' serum but disappear slowly from the patient's circulation on a GFD[126]. Extraintestinal CD symptoms may be associated with immunoglobulin A (IgA) deposits on extracellular tTG in the liver, kidney, lymph nodes and muscles of CD patients[126].

The toxic peptides, such as the 19-mer, trigger an innate immune response[101], characterized by the production of IL-15 by epithelial cells and lamina propria dendritic cells[127]. There is some evidence that this response is a generalized response in all individuals, but is amplified in CD patients (possibly due to a lower threshold to IL-15) who only get disease as a result of adaptive immune system involvement[128]. IL-15 affects the epithelial barrier, both by increasing the permeability through disruption of the tight junctions[129,130] and acting on intraepithelial lymphocytes (IELs) promoting interferon γ (IFN-γ) production as well as a potent cytotoxic activity particularly by $NKG2D^+$ cells[131,132]. Therefore, immunoadaptive peptides, like the 33-mer, can now reach the lamina propria, where they are presented by dendritic cells to gluten-specific T cells[133,134].

Other autoantigens that are normally "cryptic" can be unmasked and cause a self-aggressive immunologic response following the gliadin-initiated inflammatory process[8]. In fact, persistent stimulation by some proinflammatory cytokines (IFN-γ and tumor necrosis factor α) can cause further processing of autoantigens and their presentation to T lymphocytes by antigen-presenting cells. The mucosa is expanded by increased numbers of lymphoid cells both in the intraepithelial compartment, in which there is an increase in γδ T cells, and in the lamina propria, which is expanded by lymphocytes and plasma cells. The intestinal crypts are elongated because of an increase in dividing epithelial cells, and villi are shortened or even completely absent because of rapid loss of mature epithelial cells from the villus tip.

Intestinal epithelium function

Intestinal epithelium plays a central role in CD disease pathogenesis. It modulates the intestinal immune system that is acutely altered by gliadin. This indicates that gliadin can gain access to the basal surface of the epithelium, and therefore interact directly with the immune system, *via* both trans- and paracellular routes of absorption (Figure 3).

Retrotranscytosis

The protected retrotransport of secretory IgA into the intestinal lumen *via* the transferrin receptor CD71, allows the entry of intact and thus harmful gliadin peptides into the intestinal mucosa by a transcellular route. The overexpression of the transferring receptor CD71 in patients with active CD by transportation of gliadin across the intestinal mucosa through retrotranscytosis of secretory immunoglobulin-gliadin complexes is shown in Figure 4[135,136].

Transcytosis of α2-gliadin-33mer (an important trigger of CD) by apical-to-basal is stimulated by IFN-γ, which is a key cytokine involved in CD immunopathogenesis[137].

Paracellular route

There have been recent hypothesis associated with non-digested gliadin absorption in the intestinal lumen during the early event in CD pathogenesis by stimulation of the innate and adaptive immune system[83,101,102]. Zonulins provide information on the regulation of intercellular tight junctions (TJs) and increased intestinal permeability[138-142]. It is released by the enterocyte upon apical exposure to α-gliadin digests[129,143]. Lammers *et al*[143] have identified that MyD88 induces release of zonulin upon gliadin binding to CXCR3 on enterocytes, as a result inducing greater epithelial permeability and subsequent paracellular gliadin passage to the gut mucosa.

After binding to its surface receptor, gluten is internalized[144] and subsequently triggers a series of intracellular events including phospholipase C and Protein kinase Ca activation and actin polymerization, which lead to the opening of TJs[139,145] through Zot/Zonulin receptor (Figure 5).

Other pathways

There are several pathways including cellular signals that may be involved in the mucosal damage in CD. Deamidation of gluten peptides by tissue tTG reinforces presentation of gluten peptides by HLA-DQ2 or HLA-DQ8 molecules of plasmacytoid dendritic cells (pDCs) to T cells, which activate gluten-reactive Th1 cells and produce high levels of proinflammatory cytokines. IL-21 is overproduced in the mucosa of CD patients, where it helps sustain T-bet expression and IFN-γ production[146]. Th1 cytokines promote increased cytotoxicity of IELs and natural killer (NK) T cells which cause apoptotic death of enterocytes by the Fas/Fas ligand system, or IL-15-induced perforin/granzyme and NKG2D-MICA signaling pathways. IFN-α released by activated pDCs perpetuates the inflammatory reaction by inducing Th1

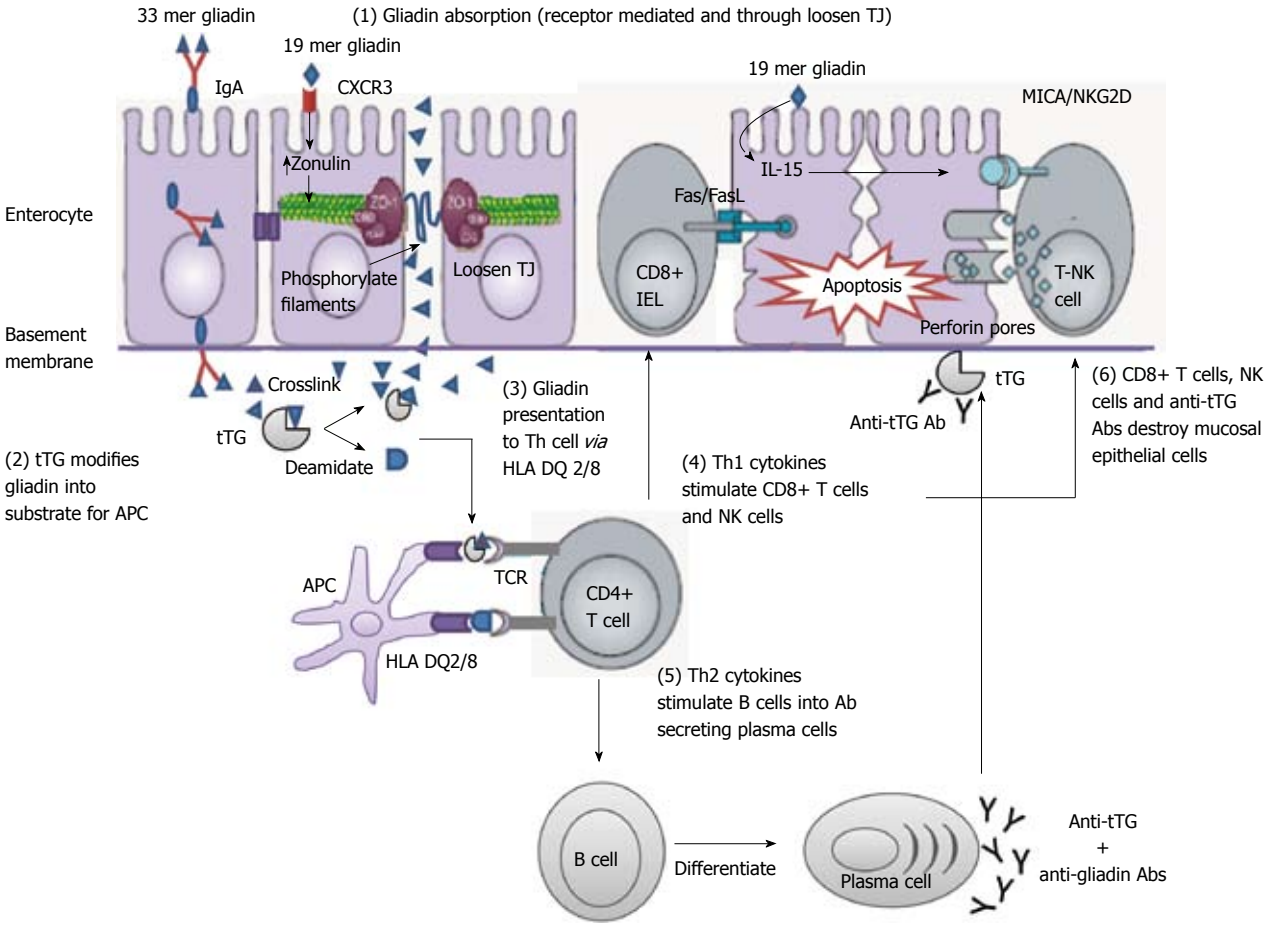

Figure 3 Mechanisms of mucosal damage in celiac disease[80] (adapted). Gliadin peptides crosses the enterocyte by paracellular tight junctions (TJ) as a consequence of increased release of zonulin leading to impaired mucosal integrity upon 19 mer gliadin binding to chemokine (C-X-C motif) receptor 3 (CXCR3) receptor, or *via* transcytosis, or retrotranscytosis of secretory immunoglobulin A (IgA) through transferrin receptor CD71. Tissue transglutaminase (tTG) deamidates or crosslinks 33 mer gliadin which is then recognized by human leukocyte antigen (HLA)-DQ2 or -DQ8 molecules of antigen presenting cell (APC). APC presents the toxic peptide to CD4+ T cells. Activated gluten-reactive CD4+ T-cells produce high levels of pro-inflammatory cytokines. T helper 1 (Th1) cytokines promote increased cytotoxicity of intraepithelial lymphocytes (IELs) and natural killer (NK) T cells which cause apoptotic death of enterocytes by the Fas/Fas ligand (FasL) system, or interleukin 15 (IL-15)-induced perforin/granzyme and homodimeric natural killer-activating receptor-major histocompatibility-class I chain-related gene A comple (NKG2D–MICA) signaling pathways. The production of T-helper2 (Th2) cytokines activate and induce clonal expansion of B cells, which differentiate into (antigliadin and anti-tTG) antibody secreting plasma cells. Interaction between with the extracellular tTG and anti-tTG-autoantibody may induce epithelial damage. TCR: T cell receptor.

cells to produce IFN-γ. IL-21 and IL-15 produced by DCs and intraepithelial cells also inhibit transforming growth factor beta signaling and regulatory T cells (Tregs) function. Additionally, the production of Th2 cytokines, Th2 cells drives the activation and clonal expansion and differentiate of B cells into plasma cells secreting anti-gliadin and anti-tissue transglutaminase antibodies[147], which interact with extracellular tTG, and may induce epithelial damage.

Hence in CD, there is impaired suppressor activity of Tregs. This defect in Tregs function could play a role in the pathogenesis of CD and in CD autoimmunity[148].

DIAGNOSIS

Approach to initial CD diagnosis

In 1970, the European Society of Paediatric Gastroenterology laid down criteria for the diagnosis of CD in children, entailing three biopsies of an initial flat mucosa in the upper small intestine, restoration of the mucosa to normal on a GFD, and a deterioration of the mucosa after gluten challenge[149]. Given the current availability of serological tests being highly sensitive and specific, the European Society of Paediatric Gastroenterology, Hepatology, and Nutrition has proposed a revised CD diagnostic protocol[150]. Based on this protocol, if the symptoms (either "classical" or "atypical") and serological tests are suggestive of CD, small bowel biopsy followed by a favourable clinical and serological response to the GFD is now considered sufficient to definitely confirm the diagnosis. In asymptomatic patients improvement in mucosal appearance may be required to confirm the diagnosis, but in majority symptomatic patients, continual abnormality of mucosa at the second biopsy is more likely to indicate slow /partial mucosal recovery[151]. This may also reflect that the site of re-biopsy (proximal small intestine) is often the last site to improve.

The current approach to evaluating CD has been modified by the advent of highly sensitive and specific serological tests. An algorithm for diagnosing CD is

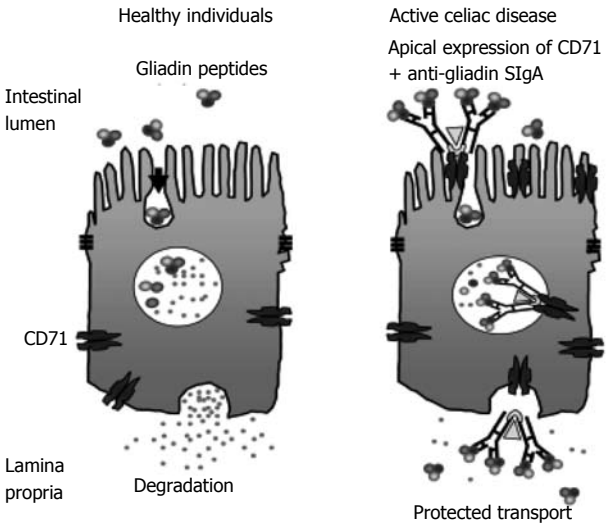

Figure 4 CD71 receptor-mediated transport of immunoglobulin A-gliadin complexes in celiac disease[136] (adapted). Gliadin bound to apically expressed CD71 receptor in active celiac disease individual allows protected transport of gliadin into the lamina propria. SIgA: Secretory immunoglobulin A.

given in Figure 6. Assays for IgA anti-tissue transglutaminase (TGA) and IgA anti-endomysial (EMA) have both the highest specificities and sensitivities, and are therefore regarded as being superior serological screening tools for diagnosis of CD[152].

Initial CD evaluation is based on a combination of positive CD-specific serological tests, histological findings in the intestinal biopsy, CD-predisposing gene encoding HLA DQ2 or DQ8, family and medical history of CD, and clinical or histological response to GFD[26,80]. However, CD diagnosis can be challenging in some non-responsive patients to GFD[7]. Practically all patients with CD carry HLA-DQ2 or HLA-DQ8. Thus the absence of these gene pairs reflects a very high negative predictive value for CD and should prompt consideration of other causes of small bowel-related symptoms and pathological changes[153,154]. Positive TGA or EMA at initial diagnosis of CD or at any time in the clinical course of the disease helps to confirm the diagnosis of CD because of their excellent specificities of over 99% when small bowel villous atrophy is present on biopsy[155].

However, false positive serological assays may also occur[156], in liver disease and small-bowel inflammation[157], so documentation of gluten sensitivity is important. A combination of biopsy and serological antibody can also be used to support diagnosis to reduce false positive results. A validated subjective Celiac Dietary Adherence Test, a patient-completed tool, can also be used in conjunction with biological markers to assess dietary adherence and disease activity in individuals with CD[158].

Diagnosis of refractory CD

The influence of noncompliance to a GFD and the substantial number of patients being undiagnosed are of greatest concern, as these factors could possibly contribute to the refractory form of CD and to the development of malignancies. These patients' CD symptoms do not revert on GFD. The first evaluation step of a potential RCD case is to confirm correct initial diagnosis of CD[7]. Sometimes neglected in this determination is the documentation of an initial and convincing response to a GFD, i.e., demonstration that the disease was truly a "gluten-sensitive" small bowel disorder. Otherwise, it may be difficult to ascertain if CD was initially present. More precise terms in this clinical setting include "sprue-like intestinal disease" or "unclassified sprue".

Some patients with developed RCD are likely to have negative TGA and EMA[6,159], demonstrating that negative CD specific serology does not exclude the diagnosis of CD. A family history of CD in first-degree relatives (especially siblings) further supports the diagnosis of CD in patients, having 14% positive tTG test and 10% positive EMA with an estimated prevalence of 11% where 54% had "silent" disease, most with severe intestinal villous atrophy[76]. The diagnosis of CD by histological findings or clinical improvement after GFD without confirmation with other diagnostic criteria may not be entirely reliable because CD is just one of many causes of villous atrophy. Clinical response to GFD or exacerbation after gluten re-introduction have low sensitivity of 59% and specificity of 92% for CD[160], which account to a positive likelihood ratio of 7.37 (means that individuals with positive histology upon gluten re-introduction are 7.37 times more likely to have CD than those with negative histology upon gluten re-introduction) and a negative likelihood ratio of 0.44 (means that individuals with positive histology upon gluten re-introduction are 0.44 times less likely to lack CD than those with negative histology upon gluten re-introduction). Thus, a critical review of prior tests and villous histology is crucial to determine the accuracy of a prior diagnosis of CD. Ideally, documented normalization of biopsies after a GFD and then demonstration of recurrent symptoms with histological relapse best defines refractory CD (RCD). Obviously, this is not always possible.

RCD is believed to affect approximately 5% of patients with CD. It is subdivided into types I and Ⅱ, with normal and aberrant (expressing cytoplasmic) CD3, but lacking surface expression of the T-cell markers CD3, CD4, CD8[161], and the T-cell receptor, intraepithelial T lymphocytes in the small intestinal mucosa, respectively[162]. Enteropathy-associated T-cell lymphoma (EATL) occurs in more than half of patients with RCD Ⅱ within 4-6 years after RCD Ⅱ diagnosis, and is the main cause of death in this group of patients[76,163]. RCD type 1 only rarely evolves into EATL. Recent data indicate a relative risk for patients with (untreated) CD to develop EATL[164,165].

DIAGNOSTIC TESTS

Serological tests

HLA typing: The contribution of HLA type to the genetic risk for CD has been variously estimated at

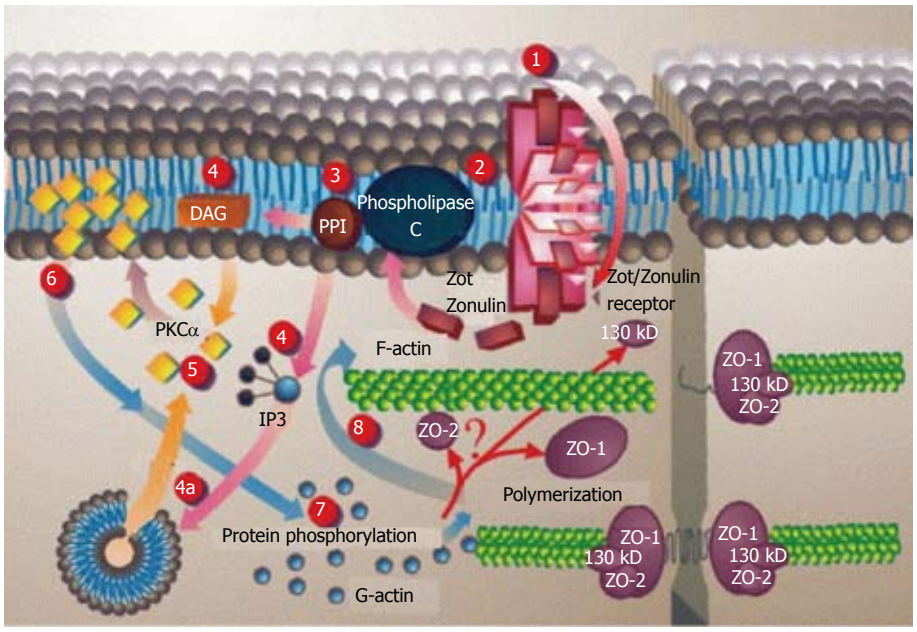

Figure 5 Proposed Zot intracellular signal mediated opening of intestinal tight junctions[146] (printed with permission). 1: Zot interacts with a specific Zot/Zonulin intestinal surface receptor; 2: Leading to protein internalization; 3: Activation of phospholipase C; 4: Hydrolyzes phosphatidyl inositol to release inositol 1,4,5-tris phosphate (PPI-3) and diacylglycerol (DAG), either *via* DAG or (4a) through the release of intracellular Ca^{2+} *via* PPI-3; 5: Protein kinase C alpha (PKCα) is then activated; 6: Membrane-associated, activated PKCα catalyzes the phosphorylation of target protein(s); 7: With subsequent polymerization of soluble G-actin in F-actin; 8: This polymerization causes the rearrangement of the tight junctions (TJ) filaments and displacement of proteins [including zonula occludens-1 (ZO-1)]. As a result, intestinal TJ becomes loosened. IP3: Inositol trisphosphate.

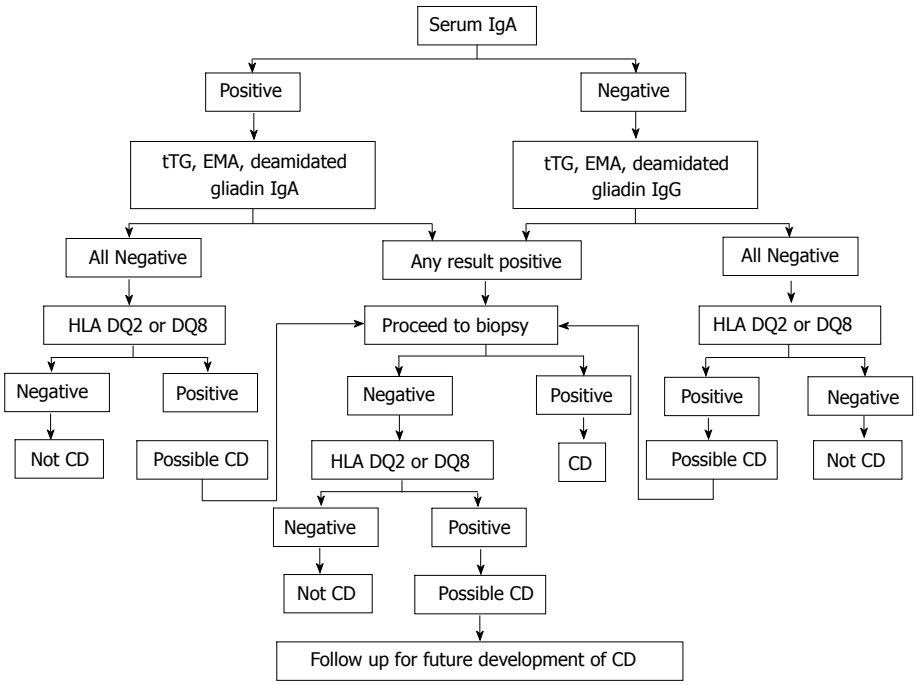

Figure 6 Celiac disease diagnostic testing algorithm (adapted from Mayo Medical Laboratories, Mayo Foundation for Medical Education and Research). IgA: Immunoglobulin A; IgG: Immunoglobulin G; tTG: Tissue transglutaminase; EMA: Endomysial; HLA: Human leukocyte antigen; CD: Celiac disease.

30%-50%[20,21]. Many of the polymorphic genes are involved in susceptibility to CD encode products that influence the immune response upon gluten ingestion, as shown for the HLA-linked genes[22]. Although Non-HLA genes contribute more than HLA genes to the genetic background of CD, each of them adds only a minor contribution to the disease development.

There is strong association between CD and the presence of HLA DQA1*0501-DQB1*02 (DQ2) and DQA1*0301-DQB1 [0302 (DQ8) haplotypes. Approximately 90% to 95% of patients with CD carry DQ2 and those patients that are negative for HLA-DQ2 are usually positive for HLA-DQ8[166,167], indicating a strong genetic risk for the disease[100]. Several studies also have confirmed that the absence of HLA-DQ2, HLA-DQ8, or both virtually excludes the diagnosis of CD[168-170]. However, the modest sensitivity (HLA-DQ2, 70%-99.8%; HLA-DQ8, 1.6%-38%) and specificity (HLA-DQ2, 69%-77%; HLA-DQ8, 77%-85%) of the test means that a positive result is not sufficient to diagnose the disease[having a low positive predictive values (HLA-DQ2, 6.3-18; HLA-DQ8, 0.28-8.1) and likelihood ratios (HLA-DQ2, 2.25-4.33; HLA-DQ8, 0.07-2.53)][171]. Even the presence of HLA-DQ2 or HLADQ8 in patients with positive serologic test results is strongly suggestive but not pathognomonic for CD. Antibody screening to identify participants with preclinical CD may be reduced by preselecting HLA risk group from the large populations with long-term follow-up for CD[172]. Hence HLA-DQ genotyping could be included in the algorithm of selecting large populations prospec-

Table 6 Operating characteristics of serological markers to detect the celiac disease in adults[178] (adapted)

Serological tests	Sensitivity	Specificity	Predictive value		Likelihood ratio	
	95% CI (%)	95% CI (%)	Positive	Negative	Positive	Negative
IgG AGA	57-78	71-87	0.2-0.9	0.4-0.9	1.96-6	0.25-0.61
IgA AGA	55-100	71-100	0.3-1.0	0.7-1.0	1.89-∞	0-0.63
IgA EMA	86-100	98-100	0.98-1.0	0.8-0.95	43-∞	0-0.14
IgA TGA	77-100	91-100	> 0.9	> 0.95	8.55-∞	0-0.25
IgA TGA and EMA	98-100	98-100	> 0.9	> 0.95	49-∞	0-0.02

IgG: Immunoglobulin G; IgA: Immunoglobulin A; AGA: Anti-gliadin antibodies; EMA: Endomysial; TGA: Transglutaminase.

tively screened for CD.

Antibody level: Several serum antibodies have been used to initially evaluate patients with suspected CD, monitor adherence and response to GFD, and screen asymptomatic individuals. Anti-gliadin antibodies (AGA) detection has low sensitivity and specificity, leading to high false-positive rate in patients[173]. Recent reports of deamidated gliadin peptide AGA (DGP-AGA) have suggested a much improved accuracy[174]. The sensitivity and specificity for IgA DGP-AGA is 84.3% and 79.8%, whereas for IgG DGP-AGA the sensitivity and specificity are 82.3% and 98.9%, respectively[175]. As shown in Table 6, EMA and TGA have been found to be superior to AGA and gives highest sensitivity and specificity of greater than 95% when used in combination[173,176,177]. EMA testing, however, produces a subjective and highly observer-dependent result, whereas TGA testing is quantitative.

Small intestinal mucosal biopsy

Histopathological analysis: Although the diagnosis of CD can be suspected on clinical or laboratory grounds, or as a result of serological tests, histology of the proximal small intestinal mucosa is still the diagnostic gold standard and must always be performed. Small intestinal histopathology of CD biopsy samples are characterized by typical architectural abnormalities. Marsh[178] has pioneered the theory of a sequence of progression of the CD lesion in the small intestinal mucosa.

According to the modified Marsh classification: normal mucosa is classified Marsh 0, intraepithelial lymphocytosis as Marsh Ⅰ, intraepithelial lymphocytosis and crypt hyperplasia as Marsh Ⅱ, and intraepithelial lymphocytosis, crypt hyperplasia and villous atrophy as Marsh Ⅲ[179]. Later the Marsh-Oberhuger system was developed, where stage 3 was split into three sub stages (a, b and c)[178,180]. The Marsh-Oberhuber classification was based on a 6-stage grading, namely (1) type 1 infiltrative lesions, characterized by normal mucosal architecture with an increased number of IELs; (2) type 2 hyperplastic lesions, characterized by an increase in crypt depth without villous flattening; (3) type 3a, 3b, and 3c destructive lesion, characterized by mild, marked, and complete villous flattening, respectively; and (4) type 4 hypoplastic lesions, characterized by villous atrophy with normal crypt height and IEL count.

Considering the broad spectrum of lesions possibly present in CD, the Marsh-Oberhuber system is undoubtedly valid under optimal clinical conditions, but the considerable number of diagnostic categories involved makes it prone to a low inter-observer and intra-observer agreement.

False-positive and false negative test results may occur due to patchy mucosal damage, inter-observer variability, low-grade histopathological abnormalities and technical limitations. Hence, reliance on standard histological findings alone may result in failure to diagnose CD[181]. Several other limitations may be evident in high-volume, service-oriented laboratories with limited attention to quality control. Poorly oriented biopsies fixed in the endoscopy suite may be prone to difficult interpretation. Inter-observer variation in pathological interpretation may occur, especially if there is limited access to a pathologist with expertise focused on interpretation of small intestinal biopsies. Some patients with low-grade histopathological abnormalities (Marsh Ⅰ/Marsh Ⅱ) can present with gluten-dependent symptoms or disorders before overt villous atrophy occurs. Furthermore, some patients with isolated intraepithelial lymphocytosis (Marsh Ⅰ), who are not clinically suspected of having CD, may develop CD during follow-up[182]. Although the mucosal changes in CD are thought to develop gradually, whether minor mucosal lesions in asymptomatic patients indicate CD in an early stage is not yet clear[183].

In case of strong clinical suspicion of CD, duodenal biopsy must be performed regardless of serological analysis[184]; in cases of low suspicion of disease or screening, duodenal biopsy probably only needs to be performed in seropositive patients. Hence, the new system for routine use of simplified grading system with uniform diagnosis and increase validity of the pathologic diagnosis of CD was developed by using only three categories (A, B1 or B2) with A representing normal villous with lymphocytic infiltration and B1 and B2 representing partial and complete villous atrophy, respectively[185]. The new proposed grading system classified the CD lesions into non-atrophic (grade A) and atrophic (grade B)[186]. Grade A was characterized by the isolated increase of IELs (> 25/100 enterocytes)[187], whereas grade B was split into B1, in which the villous/crypt ratio is less than 3/1, with still detectable villi, and B2, in which the villi are no longer detectable. A comparison between the Marsh-Oberhuber and the new

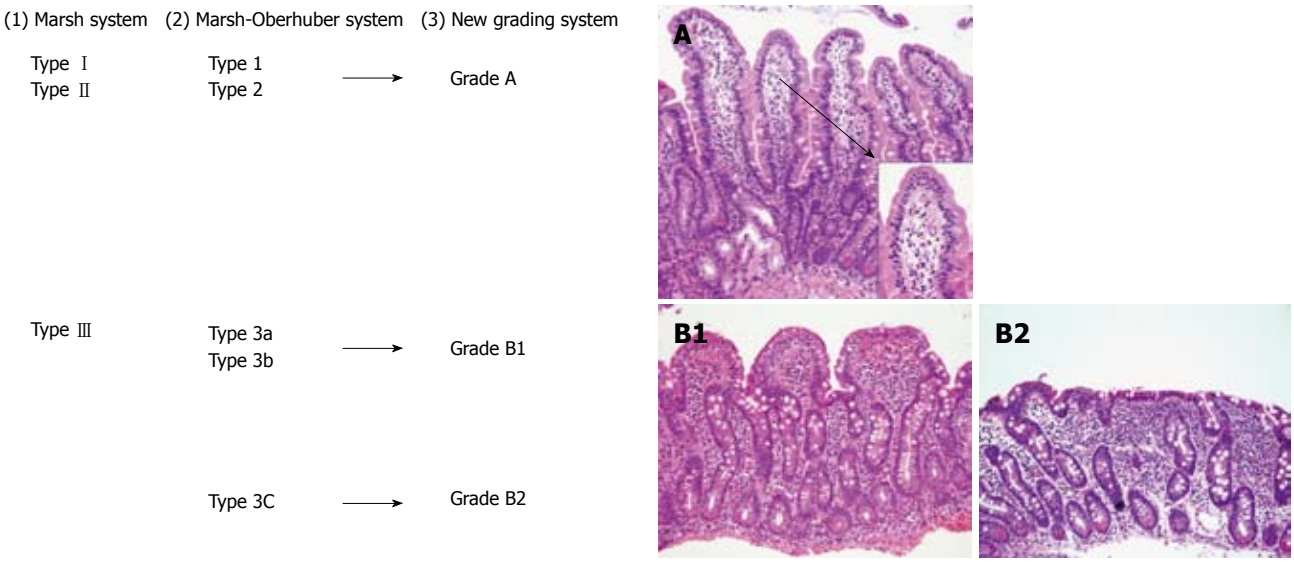

Figure 7 A comparison between the Marsh classification for celiac disease. 1: Marsh-Oberhuber; 2: Grading system for celiac disease, and the new grading system; 3: Representative pictures of the grades A (original magnification, 20×; insert, 60×), B1 (20×), and B2 (20×), proposed in the new grading system. An alternative classification may simply describe "mild", "moderate" or "severe (flat)" architectural changes[186] (printed with permission).

Table 7 Factors that support the diagnosis of celiac disease in patients with an increased density of intraepithelial lymphocytes but no villous shortening[194] (printed with permission)

Family history of celiac disease	15% of first-degree relatives are affected
Concomitant autoimmune conditions	Risk of coeliac disease approximately 5-fold
Increased density of γδ+ IELs	Sensitivity 0.84, specificity 0.91
Increased density of villous tip IELs	Sensitivity 0.84, specificity 0.95
HLA DQ2 or DQ8	High sensitivity, low specificity Negative predictive value high
Gluten dependence	Should be ascertained by gluten challenge or gluten-free diet

IELs: Intraepithelial lymphocytes; HLA: Human leukocyte antigen.

grading criteria is shown in Figure 7. Figure 7C represents pictures of the grades proposed in the new histologic grading criteria.

Recently, quantitative measurements of villous height, apical and basal villous widths, and crypt length (morphometry) have been used to determine changes in duodenal morphology, particularly after the introduction of a GFD, in correlation with Marsh grade, self-reported adherence to GFD, and changes in serology. GFD resulted in increase in villous area and a progressive decrease in crypt length, with a plateau after 6-12 mo and mean villous area half that of control subjects[188].

Intraepithelial lymphocyte: The presence of aberrant IELs appears to be a reliable prognostic marker to differentiate between RCD type I and type Ⅱ patients, with characteristic normal and aberrant IELs, respectively. IELs are considered aberrant when there is cytoplasmic CD3 expression, but no expression of surface CD3, CD4 and CD8 T-cell markers[189,190]. The current methods for double CD3/CD8 T cell receptor clonal from intestinal tissue can be done by immunohistochemistry, polymerase chain reaction or flow cytometry[161,162,189]. The presence of these IELs is associated with a significant increase in EATL development[161,163,191,192]. Increased IEL's may be used to support or exclude diagnosis of CD, and may be useful for follow up as mentioned in Table 7[193].

In 95% of non-refractory CD and control patients, the highest percentage of aberrant T-cells in duodenal biopsy specimens is in agreement with the cut-off of the % T cells which are aberrant. Such a cut-off has been previously suggested in the RCD group based on the clinical observation that none of the RCD patients with less than 20% aberrant T-cells eventually developed EATL[163]. Clonal T-cell population can be found in the intestinal mucosa of RCD patients, which relates to the development of EATL[161,194]. Immunophenotyping using flow cytometry[162], gives significant higher negative predictive value and sensitivity (both 100%) for aberrant T-cells were found with regard to EATL development in RCD, when compared to clonality in a duodenal biopsy specimen (75% and 78%, respectively). The positive predictive values (59%) and likelihood ratios (1.85) of these tests for EATL development in RCD are almost comparable.

Aberrant T-cells is quantified by flow cytometry is well suited to identify RCD patients at risk for EATL as it has a higher predictive value and sensitivity than T-cell clonality analysis of duodenal biopsy specimens. A cut-off value of 20% appears reliable for early risk stratification[159], and targeted therapeutic options in RCD patients[6,27,195]. This is particularly important since once overt T-cell lymphoma has developed, treatment outcome and survival are very poor[159,196]. Additionally, quantification of aberrant T-cells is useful for the subsequent follow-up of treated RCD Ⅱ patients[27].

Table 8 Future therapeutic approach for celiac disease treatment

Mechanism		Therapeutic agent	Stage of study
Hydrolysis of toxic gliadin	ALV003	Glutenenases and endoprotease	Phase Ⅱ
	AN-PEP	Prolyl endopeptidase	Phase Ⅱ
		Lactobacilli	Discovery
	VSL#3	*Lyophilised bacteria*, including *Bifidobacteria*, *Lactobacilli* and *Streptococcus salivarius*	Discovery
Prevention of gliadin absorption	Larazotide	Hexapeptide derived from zonula occludens toxin of *Vibrio cholera*	Phase Ⅱ
		Synthetic polymer poly (hydroxyethylmethacrylate-co-styrene sulfonate)	Discovery
		Anti-gliadin IgY	Discovery
tTG2 inhibitor		Dihydroisoxazoles	Discovery
		Cinnamoyltriazole	Discovery
		Aryl β-aminoethyl ketones	Discovery
Peptide vaccination	Nexvax2	Three deamidated peptides derived from wheat α-gliadin, ω-gliadin and β-hordein	Phase Ⅰ
		Human hookworm (Necator americanus) inoculation	Phase Ⅱ
Modulate immune response		HLA-DQ2 blocker	Discovery
		Interleukin blocker	Discovery
		NKG2D antagonist	Discovery
Restore intestinal architecture		R-spondin-1	Discovery

tTG2: Tissue transglutaminase 2; PEP: Prolyl endopeptidases; NKG2D: Homodimeric natural killer-activating receptor; HLA: Human leukocyte antigen; IgY: Immunoglobulin Y.

Useful background for the diagnosis of CD: The HLA class Ⅱ molecules DQ2 and DQ8 are required for but are not sufficient for the development of CD: 50% of Americans are positive of one of those molecules, but only 1% develop CD. Negative HLA DQ2 or DQ8 may rule out CD as a cause of the enteropathy; IgA TGA serology is > 95% sensitive for CD, especially when there is a high titre, but false positive tests can occur; Anti-gliadin antibodies have a relationship high false negative rate, and have been replaced by IgG DGP assays that appear to have a sensitivity compared to TGA; The endoscopic features of CD (scalloping of mucosal folds, less prominent folds, fissules, and a nodular/mosaic pattern) are 59% sensitive but 92% specific for CD. For example, other small bowel disorders, including Crohn disease in the duodenum, may cause mucosal scalloping and other endoscopic features of CD.

TREATMENT

Existing treatment

GFD: Currently, the only effective treatment available for CD individuals is a strict life-long GFD[197]. In reality, total avoidance of gluten intake is extremely difficult, due to hidden gluten from food contamination[198]. For safety purposes, United States Food and Drug Administration has set the limit (August 2011) of < 20 ppm gluten (equivalent to 10 ppm gliadin) for gluten-free foods. The total daily consumption of gluten-free foods must be taken into account as it may exceed the tolerable limit of each celiac individual. It has been estimated that the threshold of prolonged gluten ingestion in some CD individuals may be lower than 50 mg/d[199]. However, some CD individuals can conceivably be more sensitive. The presence of hidden gliadin in contaminated food products represents an imminent risk for celiac consumers, because of long-term effect of regular ingestion of small amounts of gliadin[200], on causing positive tTG and characteristic small bowel biopsy.

Gluten modification: Approaches to modify dietary gluten have been made to render gliadin non-toxic, since it is a non-invasive approach to CD patients. This approach has been less appealing due to loss of baking characteristic, public refusal for genetically modified crops, contamination of genetically modified crops with gluten contained crops grown nearby and heterogeneous uncharacterised immunostimulatory epitopes in gluten, and difference among patients response to epitopes and gluten levels[201].

A greater understanding of the pathogenesis of CD allows alternative future treatments to be designed. A number of preliminary studies have been published that illustrate from a conceptual perspective future possible approaches that may be pursued in more detail (Table 8).

FUTURE TREATMENT APPROACHES

Hydrolysis of toxic gliadin peptide

Prolyl endopeptidases: Prolyl endopeptidases (PEPs) are endoproteolytic enzymes expressed in micro-organisms and plants. These enzymes cleave proline-rich gluten to smaller peptides that are ready for digestion by intestinal brush-border enzymes (aminopeptidases and carboxypeptidases). Limited efficiency was found, since PEP required 3 h preincubation with gluten containing foods to achieve full detoxification of peptides and to prevent intestinal transport of active gluten fragments[202]. This is unlikely to be achieved by co-administration of PEP and gluten-containing diet.

A two-stages cross-over phase Ⅱ clinical trial was performed using asymptomatic CD patients eating, a slice of bread daily and a slice of bread pre-treated with PEP daily[203]. After 2 wk of PEP treated gluten challenge, majority of patients did not develop malabsorption, measured by faecal fat excretion and D-xylose

malabsorption tests. The tests likely lacked the necessary sensitivity to assess minor malabsorption resulting from active CD, since no histological confirmation was performed to determine deterioration in the Marsh grading[201]. When PEPs were consumed as jam spread on a slice of gluten-containing bread by CD patients, villous blunting was seen in small bowel biopsy histological evaluation in most patients[204]. Further studies are needed to determine the appropriate dose of enzyme and time of administration relative to the quantity of ingested gluten.

ALV003: ALV003, a mixture of two glutenases, an endoprotease from germinating barley and PEP, was pretreated with wheat flour and tested in CD patients[205]. Symptoms typically associated with gluten ingestion were not significantly reduced by ALV003 pre-treatment, but ALV003 abolished immune responses induced by gluten in CD patients. A randomized controlled phase Ⅱa clinical trial has been performed where CD patients received either ALV003 or placebo daily for 6 wk at the time of 2 g gluten contained bread. This proof-of-concept study demonstrated that ALV003 can attenuate gluten-induced small intestinal mucosal injury in CD patients[206]. After six weeks period, biopsies proved lower small intestinal mucosal injury in patients treated with ALV003 than placebo-treated patients despite of persistent intestinal inflammation in many patients on a strict GFD. Placebo-treated patients were found to have suffered more adverse events, most commonly including abdominal distention, flatulence, eructation, abdominal pain and diarrhea[206].

Lactobacilli: Lactobacilli added to sourdough for fermentation are able to lyse the proline-/glutamine-rich gluten peptides and thus decrease immunotoxicity[207-210]. A mixture of fermented wheat flour with oat, millet and buckwheat allows sourdough bread to retain its baking characteristics. A pilot study in patients with CD suggested that this bread was well tolerated[209]. However, these patients were challenged for only 2 d, which is clearly not sufficient to draw any firm conclusions. Hence, another 60-d diet of fully hydrolyzed wheat flour with sourdough lactobacilli and fungal proteases (8 ppm residual gluten; n = 5) was further studied. The pretreated flour was rendered non-toxic by serological, morphometrical, and immunohistochemical analysis[211]. A larger group of subjects in the trial and palatability of digested flour baked products needs to be taken into consideration.

VSL#3: VSL#3 is a probiotic containing lyophilised bacteria, including bifidobacteria (*Bifidobacterium longum, Bifidobacterium infantis* and *Bifidobacterium breve*), lactobacilli (*Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus delbrueckii subsp., Lactobacillus bulgaricus* and *Lactobacillus plantarum*) and *Streptococcus salivarius subsp., Thermophiles*. It is used to hydrolyse gliadin peptides in pre-treated flour and tested for efficacy in rat intestinal cell line and celiac jejunal biopsies[207]. VSL#3 pre-digested gliadins did not show an increase of the infiltration of CD3+ intraepithelial lymphocytes and caused a less pronounced effect on intestinal mucosa permeability (determined by lower F-actin rearrangement and zonulin release). Hence, VSL#3 may have importance during food processing to produce pre-digested gluten-free products.

Prevention of toxic gliadin peptide absorption

Larazotide: Larazotide (AT-1001, Alba Therapeutics, Baltimore, MA), is a synthetic hexapeptide derived from Zonula Occludens toxin of Vibrio cholera[212]. It is used to inhibit the opening of tight junctions of the small intestine epithelial cells. Clinical trial phase I in CD patients suggested that Larazotide therapy is well tolerated by patients and reduces intestinal barrier dysfunction, pro-inflammatory cytokine production, and gastrointestinal symptoms in CD individuals after gluten exposure[213]. Encouraging results were obtained from a 6-wk phase Ⅱb trial in terms of symptoms and antibody titers[214], showing larazotide acetate a promising drug candidate. This drug inhibits the paracellular route of gliadin absorption through tight junctions, which is not the only mechanism of gliadin absorption. Indeed, gliadin may gain access to the mucosa through transcellular pathways in addition to paracellular route[135,137]. Hence, this strategy might be best exploited in combination with other treatments.

Synthetic polymer poly (hydroxyethylmethacrylate-co-styrene sulfonate): Poly (hydroxyethylmethacrylate-co-styrene sulfonate) [P (HEMA-co-SS)] forms supramolecular particles upon gliadin complexation in gastric and intestinal conditions[215,216], and deteriorates gliadin's effect on epithelial cells[217]. This complexation decreases the effect of gastrointestinal (GI) digestive enzymes on gliadin absorption, and thus the formation of immunogenic peptides is reduced. Gluten-sensitive HLA-HCD4/DQ8 mice co-administered with P (HEMA-co-SS) showed attenuated gliadin-induced changes in permeability and inflammation[217]. Low side effect, cost and possibility to be taken, occasionally with gluten-containing food, makes it an attractive option. Further investigation of the mechanisms of action and its interaction with human tissues is required before its efficacy is investigated in human trials[218].

Anti-gliadin egg yolk antibody: Oral antibody passive immunotherapy may be of value due to the advantages of reduced cost, ease of administration, and potential to treat localized conditions in the gastrointestinal tract[219]. Among antibodies, chicken egg yolk immunoglobulin (IgY), is ideal for passive immunotherapy, as it may be readily obtained in large quantities from egg yolk, presenting a more cost-effective, convenient, and hygienic alternative to mammalian antibodies. Oral immunotherapeutic IgY is a promising alternative to neutralize gliadin in the GI tract and prevention it from absorption[220]. Mannitol contained antibody preparation is highly resistant against GI enzymes and proved to effectively

neutralized gliadin under simulated GI conditions in the presence of food. *In vivo* study; BALB/c mice fed with IgY formulation and gliadin ratio of 1:5 (w/w), demonstrated that gliadin absorption in the gastrointestinal tract was minimal at < 1%[221]. Further investigations in CD patients is requires to prove its efficacy and determine dosing regimen of antibody relative to the amount of gliadin ingestion.

Blockage of selective deamidation of specific glutamine residues by tissue transglutaminase 2 inhibitor

Transglutaminases (a family of eight enzymes) have diverse functions in human and are involved in several biological and pathological processes[222]. tTG2 is an enzyme that has a pro-inflammatory effect and increases the immunostimulatory epitopes present in the lamina propria of the small intestine. Blockage of tTG2 may be a promising approach to inhibit the inflammatory process upon gluten ingestion. There are two essential classes of tTG2 inhibitors; irreversible and reversible inhibitors[223]. Irreversible inhibitors form a stable covalent bond with this enzyme, and thus prevent deamidation of gliadin peptides[223,224]. Reversible inhibitors are more desirable to minimize possible side effects. These include aldehyde-bearing tTG modulators[225], cinnamoyl triazole derivatives[226], and the highly specific modified peptide targeting the active cysteine site of tTG2[227]. Since a few gluten T-cell epitopes can be recognized without being deamidated by tTG2[228,229], this approach will not inhibit the innate response[101], or the immune response induced by non-deamidated peptides[82]. To be able to use tTG2 inhibitors clinically, it is critical to design highly specific inhibitors, since all human tTG share high sequence homology.

Vaccine application to restore immune tolerance towards gluten

Autoimmune enteropathy in CD has been proposed to be due to impairment of immunoregulatory mechanisms that controls oral tolerance. Systematic peptide mapping of T-cell was performed to determine gliadin reactive epitopes recognized by approximately 90% of CD patients. A clinical trial phase I study has been initiated as Nexvax2® (Nexpep Pty, Ltd., Australia) peptide vaccine-containing mixture of immunotoxic α- and ω-gliadins and B-hordein[230].

Engineered Lactococcus lactis secreting a DQ8-restricted gliadin peptide administered orally[231], or recombinant α-gliadin in HLA-DQ8 administered intranasally in transgenic mouse model[232], have been studied to modulate immune response to gluten. However, it is difficult to appreciate how the vaccine or the intranasal peptide administration can modulate the Tr1 response. More work is needed to assess the effect of these therapies on the spectrum of gluten peptides presented to the gut.

Dermal inoculation of human hookworm (*Necator americanus*) has also been used to modulate the immune response to gluten[233]. A phase Ⅱ trial with CD patients suggested that hookworm infection on its own may not obviate the necessity for a restricted diet in CD, but appears to be safe and might impact on immune pathology[234]. Here in, hookworm infection is expected to reduce gluten sensitivity and immune reactivity.

Modulation of immune response to dietary gliadin

HLA-DQ blocker: HLA-DQ blocker is used to block the binding sites of HLA-DQ2 or DQ8 for it to be unrecognized by T cells as well to block the presentation of the antigen. This is not a new concept that was developed without much success to treat type 1 diabetes mellitus and rheumatoid arthritis, due to difficulties in effective drug delivery[235,236]. By amino-acid substitution of gliadin T-cell stimulatory sequence, the epitope can be converted to an agonist or antagonist, abolishing the inflammatory cascade[237]. IFN-γ production by peripheral blood lymphocytes was prevented when either an alanine or lysine amino acid was substituted through the immunodominant α-gliadin peptide, corresponding to the peptide's anchor to the HLA-DQ cleft[238]. To develop this as a new therapeutic agent, more studies need to be performed, looking at the mass T-cell action of the gut towards these modified peptides.

Interleukin blocker: Modulation of cytokine production has been evaluated for the treatment of several autoimmune diseases, although their side effects may be severe. Modulation of proinflammatory IL-15 and anti-inflammatory IL-10 cytokines has been suggested to influence the immune balance between tolerance and autoimmunity[127,239-241]. Blocking IL-15 may promote maintenance of epithelial integrity, limit epithelial destruction, leading to decreased passage of dietary gliadin.

NKG2D antagonists: MICA molecules, strongly expressed on active CD epithelial cell surface upon gliadin challenge[132], interact with the NKG2D-activating receptor on human natural killer cells and CD8 T cells, leading to villous atrophy due to an IEL-mediated damage to enterocytes[131,132]. Thus, NKG2D antagonists[131] and anti-NKG2D antibodies[242], have been proposed as therapeutics in CD.

Restoration of intestinal architecture by R-spondin-1

R-spondin-1 is an intestinal mitogen, shown to stimulate crypt cell growth, accelerate mucosal regeneration and restore intestinal architecture in mouse models of colitis[243]. This agent has yet to be tested in human to be considered as a therapeutic agent in CD.

CONCLUSION

CD has been kept in the dark for decades with very little known about what is a relatively common medical condition. It is only recently that we have greater understanding of its prevalence, diagnosis and pathogenesis, which has supported the development of new therapeutic approaches to treat CD. There are several future

Table 9 Key points from recent findings

Cause
Environmental (gluten) and genetic factors (HLA and non-HLA genes)
Prevalence
0.5%-1% worldwide in normal at-risk population
Higher risk in the population with diabetes, autoimmune disorder or relatives of CD individuals
Pathogenesis
Gliadin gains access *via* trans- and para-cellular routes to the basal surface of the epithelium, and interact directly with the immune system
Types of CD symptoms: "typical" or "atypical"
Diagnosis
Positive serological (TGA or EMA) screening results suggestive of CD, should lead to small bowel biopsy followed by a favourable clinical and serological response to the GFD to confirm the diagnosis
Current treatment
Strict life-long GFD
Alternative future CD treatments strategies
Hydrolysis of toxic gliadin peptide
Prevention of toxic gliadin peptide absorption
Blockage of deamidation of specific glutamine residues by tissue
Restoration of immune tolerance towards gluten
Modulation of immune response to dietary gliadin
Restoration of intestinal architecture

HLA: Human leukocyte antigen; CD: Celiac disease; EMA: Endomysial; TGA: Transglutaminase; GFD: Gluten-free diet.

directions to follow to treat CD, which if successful will supplement or even replace the current only effective treatment, the use of a GFD. A greater understanding of the pathogenesis of CD allows alternative future CD treatments to hydrolyse toxic gliadin peptide, prevent toxic gliadin peptide absorption, blockage of selective deamidation of specific glutamine residues by tissue, restore immune tolerance towards gluten, modulation of immune response to dietary gliadin, and restoration of intestinal architecture (Table 9).

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