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Review article

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CELIAC DISEASE: A MULTI-FACETED MEDICAL CONDITION

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Celiac disease (CD) is a systemic condition of autoimmune origin, affecting genetically predisposed individuals who at some point lose tolerance towards dietary gluten. Prevalence in the general population is 0.5 – 1%, with a higher frequency in women. The most important environmental factor for CD is ingestion of specific gluten peptides. It triggers a sequence of molecular events, involving the intestinal permeability and the immune system, which ends in damage of the intestinal mucosa. A number of studies have demonstrated the correlation between the intestinal microbiota and celiac disease. MicroRNAs through their regulatory role on gene expression have been implicated in the pathogenesis of CD and suggested as potential biomarkers. In the pediatric and adult population, CD displays different clusters of clinical symptoms. Persistent diarrhea, abdominal pain and involuntary weight loss are the classic symptoms of CD. In the majority of cases diagnosis relies on the combination of serum autoantibodies (anti-transglutaminase and anti-endomisium IgA) and duodenal biopsy showing villous atrophy, crypt hyperplasia and intraepithelial lymphocytes. Observance of a lifelong gluten-free diet, which interrupts the immune response to gluten peptides, is the only effective treatment of CD.

Key words: *celiac disease, immune response, microRNAs, long non-coding RNAs, anti-transglutaminase and anti-endomisium antibodies, gluten-free diet*

INTRODUCTION

Celiac disease (CD) is an autoimmune condition that occurs in people with a genetic predisposition in which dietary gluten triggers specific serological and histological changes (1). Initially, the disease was described in children and for a long time it was considered a pediatric disease. Subsequent research has demonstrated that it may occur at any age (2, 3). The epidemiology and symptomatology of CD have changed significantly in the last years, with a marked increase of its prevalence in adults (4). Changes have also been noted regarding the clinical presentation of CD, from the typical forms of malnutrition to forms with few atypical symptoms such as anemia or osteoporosis and even asymptomatic forms diagnosed by screening of high-risk population groups (5-7).

Celiac disease is more frequent in women and presently its prevalence in the general population is 0.5 – 1% (1). The prevalence has increased over time and varies with sex, age, geographic location (8-10).

The purpose of this review is a deeper understanding of aspects related to the management of celiac disease: etiopathogenesis, clinical features and at-risk comorbidities, diagnostic methods, complications, treatment and follow-up in order to help general practitioners, internal medicine physicians and gastroenterologists in their clinical practice.

ETIOPATHOGENESIS OF CELIAC DISEASE

Environmental factors

The precise cause of CD is not known. Practically the disease is triggered by an excessive immune reaction at the contact of the intestinal mucosa with the gluten in some cereals; it is characterized by intestinal and/or extraintestinal manifestations (11).

Studies that have investigated the duration of breastfeeding and the timing of gluten introduction in children have shown that they have no influence on the risk of developing CD. Other risk factors associated with the onset of the disease are gastrointestinal infections, surgery, certain medications, α-interferon (11).

Genetics

The CD patient population has a certain degree of genetic clustering, which exposes relatives of CD patients to a higher disease risk (4).

Studies have detected genetic mutations that could be correlated with this disease. The genes involved are situated near the ones controlling type 1 diabetes mellitus (T1D) and are represented by the areas of chromosomes 7 – 8 in which mutations are seen (12-14).

Table 1. Classes of microRNAs involved in the pathogenesis of CD and the genes they regulate.

Upregulated microRNAs	Pathways	Target genes	Ref.	Downregulated microRNAs	Pathways	Target genes	Ref.
miR-449a	Cell proliferation and differentiation	KLF4 DLL1 LEF1 NOTCH1 NUMBL	23, 10	**miR-31-5p**	Regulatory T cell development	FOXP3	23, 64, 100
	PI3K-Akt signaling	PDPK1 AKT2 KITLG MET COL4A4 CSF1R CCND1 FGF2 FGF23 ITGB8 LAMC1 PDGFRA PRKCA RXRA VEGFA			PI3K-Akt signaling	KRAS MET COL4A4 INSR	
					Adherens junction	MET INSR TGFBR1	
miR-638	TG2 synthesis	TGM2	23, 64	**miR-192**	Adherens junction	FGFR1 INSR MAPK1 PTPRJ SORBS1 TJP1 TCF7 TGFBR1	23, 64, 100
	Wnt signaling pathway	APC2 CREBBP CCND1 FZD4 TCF7			Innate immune response	CXCL2 NOD2 MAD2L1	
miR-21-5p	Activation of inflammation	STAT3	23, 100	**miR-197**	Innate immune response	IL-18	23
				miR-551b-5p	Cell cycle	PRL3	23, 64
				miR-194-5p	Focal adhesion	PDPK1 AKT2 CRK RAP1B ARHGAP5 XIAP CCND2 DIAPH1 ITGB8 LAMC1 MAPK1 PRKCA PPP1CB	23, 64
				miR-338-3p	T cell commitment	RUNX1	23, 100

Abbreviations: KLF4, Kruppel-like factor 4; DLL1, delta like canonical Notch ligand 1; LEF1, lymphoid enhancer binding factor 1; NOTCH1, Notch homolog 1, translocation-associated (Drosophila); NUMBL, NUMB like, endocytic adaptor protein; FOXP3, forkhead box P3; PI3K, phosphoinositide 3-kinase; Akt, protein kinase B; PDPK1, 3-phosphoinositide dependent protein kinase 1; AKT2, AKT serine/threonine kinase 2; KITLG, KIT ligand; MET, mesenchymal epithelial transition; COL4A4, collagen type IV alpha 4 chain; CSF1R, colony stimulating factor 1 receptor; CCND1, cyclin D1; FGF2, fibroblast growth factor 2; FGF23, fibroblast growth factor 23; ITGB8, integrin subunit beta 8; LAMC1, laminin subunit gamma 1; PDGFRA, platelet derived growth factor receptor alpha; PRKCA, protein kinase C alpha; RXRA, retinoid X receptor alpha; VEGFA, vascular endothelial growth factor A; KRAS, KRAS proto-oncogene, GTPase; INSR, insulin receptor; TGFBR1, transforming growth factor beta receptor 1; TG2 synthesis, tissue transglutaminase 2 synthesis; TGM2, transglutaminase 2; FGFR1, fibroblast growth factor receptor 1; MAPK1, mitogen-activated protein kinase 1; PTPRJ, protein tyrosine phosphatase, receptor type J; SORBS1, sorbin and SH3 domain containing 1; TJP1, tight junction protein 1; TCF7,

transcription factor 7; Wnt, complex protein network; APC2, APC2, WNT signaling pathway regulator; CREBBP, CREB binding protein; FZD4, frizzled class receptor 4; CXCL2, C-X-C motif chemokine ligand 2; NOD2, nucleotide binding oligomerization domain containing 2; MAD2L1, mitotic arrest deficient 2 like 1; STAT3, signal transducer and activator of transcription 3; IL, interleukin; PRL3, phosphatase of regenerating liver-3; CRK, CRK proto-oncogene, adaptor protein; RAP1B, RAP1B, member of RAS oncogene family; ARHGAP5, rho GTPase activating protein 5; XIAP, X-linked inhibitor of apoptosis; CCND2, cyclin D2; DIAPH1, diaphanous related formin 1; PPP1CB, protein phosphatase 1 catalytic subunit beta; RUNX1, runt related transcription factor 1.

A genetic factor with documented risk of CD is the presence of HLA-DQ2.5 which is encoded by the DQA1*05:01 and DQB1*02:01 genes in cis position (DR3 haplotype) or trans position (DR5 and DR7 haplotypes). Therefore most patients are DR3, DQ2 positive. The majority of persons with CD express HLA-DQ2.5, approximately 90%, while approximately 20% of patients possess HLA-DQ8 (DR4, DQ8 haplotype). HLA-DQ2.2 is present in approximately 5% of patients without DQ2.5 or DQ8. In CD patients expressing HLA-DQ2.5 or DQ8, the presence of HLA-DQ2.2 may increase prevalence up to 35% (15).

Several non-HLA genes, associated with small increases in genetic risk, have been identified as well, but only in GWAS. However, about 40% of the general population carry HLA haplotypes without having the disease, which makes their presence necessary but not sufficient for its development. In total, hereditary transmission is 50% (12, 16-18).

In the last years, studies have attempted to establish the role of microRNAs in the pathogenesis of celiac disease. MicroRNAs (miRNAs) comprise a heterogeneous group of small non-coding single chain RNAs, which regulate gene expression through control of stability and mRNA translation (19-21). These miRNAs represent important factors in the differentiation and function of intestinal epithelium, regulating expression of genes in both normal and pathological states, including inflammatory and autoimmune disease. Taking into account that at present insufficient data are available on the molecular factors underlying CD, it would be indicated to assess the profile and functions of microRNAs in these patients. Studies carried out in CD patients with different clinical features have shown an alteration of microRNA expression in comparison with healthy subjects. This may suggest that further studies on microRNAs are necessary, as they may represent biomarkers differentiating patients with different clinical features. Moreover, certain microRNA profiles could contribute to individualized patient management, shifting clinical approach from general to personalized. Eventually microRNAs could elucidate the way in which epigenetic alterations contribute to the onset and evolution of CD (22, 23) (*Table 1*).

Many SNPs associated with various diseases are located within sequences of long non-coding RNAs (lncRNAs), suggesting the alteration of the secondary structure of lncRNAs as the underlying pathogenesis (24, 25). LncRNAs have been implicated in autoimmune disorders, where they show heightened expression profiles in immune-associated regions. An important part of variation seen in GWAS could be explained by lncRNAs. SNPs are known to be medically important, they affect either the sequence of lncRNAs or their expression level, but the exact mechanisms linking lncRNAs and diseases (especially immune-mediated) remain speculative (24, 26). A total of 39 non-HLA loci have been associated with CD by several GWA studies, including the Immunochip project (24, 27-29).

Of the SNPs clearly associated with CD, only 3 are found in known protein-coding exon segments, while a number of others might be situated in putative gene regions (mainly related to immune response). The effect might be explained by their localization in the proximity of cis-regulatory segments of these regions (24, 30, 31). The only SNP linked with CD and known to belong to a functionally characterized lncRNA is connected to the NF-κB pathway, which is constitutively up-regulated in CD intestinal epithelium (24, 32-34).

Studies have shown that a lncRNA named lnc13, situated in chromosome band 2q12, within the proposed but never confirmed gene IL18RAP, is associated with SNP rs917997 (24, 35-37). Lnc13 is expressed in a wide range of human cells and tissue types. Its location in the nuclei of mononuclear cells is of interest. This lncRNA is less expressed in intestinal mucosal cells of CD patients compared to controls, unlike the expression pattern of the supposedly coding IL18RAP (24, 37). In basal conditions, lnc13 is a repressor of a number of pro-inflammatory genes, including some known to be related to CD (STAT1, MYD88, IL1RA and TRAF2). Lnc13 interacts with the heterogeneous nuclear ribonucleoprotein D (hnRNPD), a nuclear AU1 rich RNA binding protein, and with histone deacetylase 1 (HDAC1), which modifies chromatin and negatively regulates transcription. Under inflammatory conditions, lnc13 is deteriorated by decapping enzyme 2 (DCP2), which results in detachment from the chromatin strands of the inhibitory complexes and induction of pro-inflammatory genes (24, 32) (*Table 2*). Lnc13 and its SNPs have been associated with other autoimmune diseases as well, such as rheumatoid arthritis, T1D and Crohn's disease (13, 24, 38, 39). In lnc13, the T allele is the risk variant for CD, while the C allele predisposes to T1D, confirming that the function of lncRNAs is cell type specific. While it is known that lnc13's SNP rs917997 is involved in CD by up-regulating immune and pro-inflammatory genes in the intestinal epithelium, other inflammatory effects in other cell lines, with links to different inflammatory pathologies cannot be excluded (24).

HCG14 function as a gene expression regulator is suggested by its disproportionate presence in the nucleus of intestinal epithelial cells. The SNP of HCG14 associated with CD relates to decreased levels of NOD1. The precise functional role of HCG14 in CD pathogenesis and its exact position in the inflammatory gene network remain to be characterized (31) (*Table 2*).

A previously characterized lncRNA (cardiac and apoptosis-related lncRNA, Carlr) has been functionally characterized with respect to CD. Carlr was identified in higher amounts in the cytoplasm of the intestinal mucosa of CD patients compared to controls. Carlr is required for an appropriate NF-κB network, and NF-κB is known to be constitutively activated in CD (40) (*Table 2*).

The lncRNA designated as AC104820.2, which is expressed in excess in biopsies of active CD compared to treated patients, is also the site of the CD-associated SNP rs1018326 (30) (*Table 2*).

As understanding of the molecular mechanisms of lncRNAs increases, it opens the perspective of lncRNAs as diagnostic indicators and potential therapeutic targets (24).

Immunology

Exposure of the upper smal bowel mucosa to gluten in susceptible people causes an increase of intestinal permeability, then an immune mediated reaction that involves the innate and the adaptive immune responses might take place (41). The main autoantigen of CD is the enzyme tissue transglutaminase (tTG) which is found in the lamina propria of the small bowel and deamidates glutamine residues in gluten to form glutamic acid. Glutamic acid is a negatively charged molecule which is

Table 2. Characterization of lncRNA types.

lncRNAs types	Up/down-regulated	Pathways	Target genes	Function	Ref.
lnc13	Down-regulated	NF-κB pathway	IL18RAP	Represses proinflammatory genes (STAT1, MYD88, IL1RA and TRAF2); Modifies chromatin and negatively regulates transcription; Induces proinflammatory genes expression.	24, 32-37
HCG14	Down-regulated		NOD1	Regulates gene expression.	31
Carlr	Up-regulated	NF-κB pathway	TNFAIP3 IL1B PTGS2	Encourages induction of NF-κB-stimulated genes.	40
AC104820.2	Up-regulated			Could be involved in pathogenesis of CD.	30

Abbreviations: lncRNAs, long non-coding RNAs; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; IL18RAP, interleukin 18 receptor accessory protein; STAT1, signal transducer and activator of transcription 1; MYD88, myeloid differentiation primary response 88; IL1RA, interleukin-1 receptor antagonist; TRAF2, TNF receptor-associated factor 2; HCG14; HLA complex group 14 (non-protein coding); Carlr, cardiac and apoptosis-related lncRNA; NOD1, nucleotide-binding oligomerization domain-containing protein 1; TNFAIP3, tumor necrosis factor, alpha-induced protein 3; IL1B, interleukin 1 beta; PTGS2, prostaglandin-endoperoxide synthase 2.

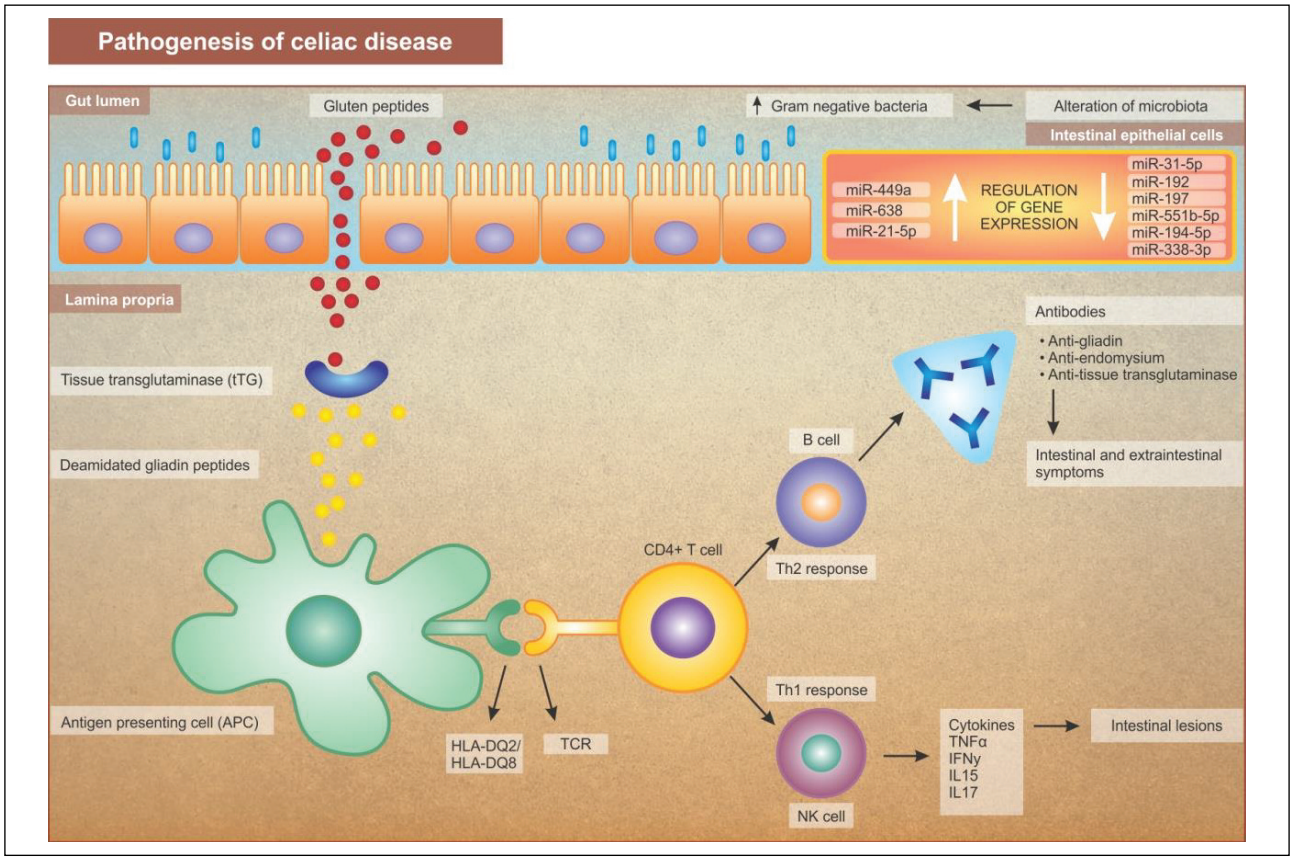

Fig. 1. Pathogenesis of celiac disease.
Abbreviations: ↑, increasing; HLA, human leukocyte antigen; TCR, T-cell receptor; CD4, cluster of differentiation 4; Th, T helper; NK cell, natural killer cell; TNFα, tumor necrosis factor alpha; IFNγ, interferon gamma; IL, interleukin.

recognized by the antigen-presenting cells that express the HLA DQ2/DQ8 receptors for T lymphocytes. Furthermore, the gluten-specific T cell response leads to the release of immunomodulators such as immunoglobulins, cytokines, interferons, tumor necrosis factor, interleukin 15 and 17 that induce intestinal lesions (42, 43).

Intestinal dysbiosis

Some studies evaluated the correlation between the intestinal microbiota and celiac disease (44). The makeup of the intestinal microbiota from duodenal biopsies as well as stools of children and adults with CD presents alterations compared to those

without CD (45). Also, differences in the intestinal microbiota were found in relation to age in subjects with and without CD (46, 47). Moreover, the differences were also noted regarding the bacterial population species in children and adults. Thus, the populations of *Firmicutes* and *Bifidobacterium* were diminished, while *Proteobacteria*, *Bacteroides* and *Escherichia coli* were increased in patients with active CD (45, 48-50). Though these data remain speculative, it might be posited that the interaction between the intestinal microbiota and the immune system differs according to the microbial composition in different age groups. This would engender different responses, related to the clinical, analytical and histological features found in adults (51).

Other studies reported that gluten-containing cereals also contain the biogenic amine histamine and the symptoms of non-celiac gluten sensitivity are similar to those found in histamine intolerance (52). One of the risk factors involved in histamine intolerance is the alteration of intestinal bacteria, also encountered in celiac disease, inflammatory bowel disease, type 1 diabetes, rheumatoid disease, obesity, cardiovascular disease. It appears that there is a correlation between intestinal histamine levels and zonulin levels in the stool, which implies a negative effect of histamine on intestinal permeability. Therefore, dysbiosis causes inflammation of the intestinal mucosa (53).

Other pathogenic factors

A smaller number of studies described the involvement of the autonomic nervous system (ANS) in celiac disease (54). The pathogenetic mechanisms that appear to be involved in ANS dysfunction are represented by autoimmune and metabolic disorders, clinical and subclinical malabsorption of microelements or vitamins, gluten toxicity, immunological reactions (neuron-specific and non-specific antibodies, cross reactions with neuronal compounds), intestinal inflammation (hyperreaction, disorders of visceral perception, local or central sensitization) (54-57) (*Fig. 1*).

CLINICAL FEATURES AND AT-RISK COMORBIDITIES

Intestinal manifestations are more common in children and may include persistent diarrhea, abdominal pain, involuntary weight loss, loss of appetite, failure to thrive, bloating, nausea, vomiting, constipation. Even though malnutrition is a frequent manifestation of CD, overweight and obesity may also be present at the time of diagnosis. Studies have shown that more than half of the adults diagnosed with CD are obese, while only 15% are underweight (1, 58) (*Fig. 2*).

The extraintestinal manifestations found are fatigue, anemia, hypoalbuminemia, hypertransaminasemia, deficiencies (vitamins D, B12, folic acid, Fe, Cu, Zn, carnitine), tooth enamel disorders (enamel hypoplasia), aphthous stomatitis, bone metabolic disorders (osteopenia, osteoporosis), abnormalities in coagulation factors (hemorrhagic diathesis due to malabsorption of vitamin K), neurological disorders (headache, epilepsy, cerebellar ataxia, peripheral and central neuropathy due to decreased absorption of vitamins B1, B6 and B12 with muscle fatigue, altered reflexes, paresthesia, up to severe motor deficits), psychiatric disorders (anxiety, depression, apathy, irritability, eating disorders, attention-deficit/hyperactivity disorders, social phobia, schizophrenia, autism), endocrine and gynecological manifestations (amenorrhea, infertility, late menarche, recurrent miscarriages, premature birth, early menopause), dermatological

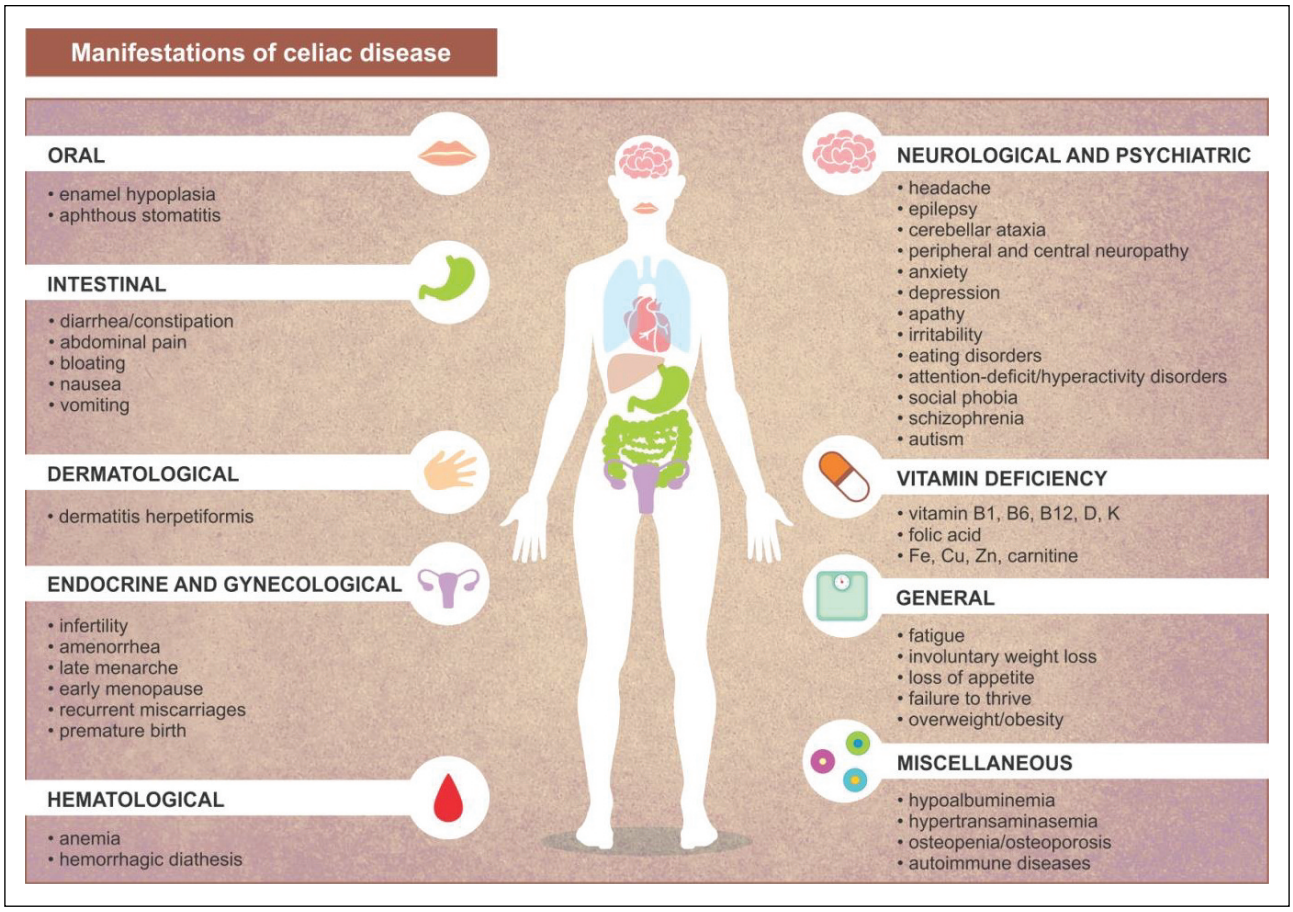

Fig. 2. Manifestations of celiac disease.

conditions (dermatitis herpetiformis), autoimmune diseases (1, 4, 59-61) (*Fig. 2*).

Types of celiac disease

Classic celiac disease includes malabsorption signs and symptoms such as diarrhea, weight loss, failure to thrive, bloating. This type is present in children and adults.

Non-classic celiac disease includes no malabsorption signs and symptoms. Patients with this type present reflux, dyspepsia, abdominal pain, vomiting, bloating, constipation or extraintestinal manifestations such as fatigue, anemia, dental enamel hypoplasia, osteoporosis/osteopenia, vitamin deficiencies, hypertransaminasemia, dermatological, gynecological, neurological, psychiatric conditions. This type is present in later childhood or adulthood.

Potential celiac disease: includes positive CD serology and normal small bowel biopsy. This type may be asymptomatic or may have classic/non-classic symptoms.

Non-responsive celiac disease: includes persistence of symptoms after 6 – 12 months on a gluten-free diet (GFD). In this type accidental gluten ingestion is the most common cause.

Refractory celiac disease (RCD): a diagnosis of refractory celiac disease (RCD) requires the persistence of symptoms in spite of a gluten-free diet for 12 months or longer, and implies the exclusion of other causes of villous atrophy. Primary resistance involves lack of response to diet from the onset, while secondary resistance occurs years or decades later, after an initial therapeutic response. Refractory CD is subdivided in type I and type II, based on the absence or, respectively, the presence of abnormal intraepithelial lymphocytes, the latter referring to the presence of an aberrant lymphocyte population. These two types are fundamentally different considering that type II resistance may develop into a low grade lymphoma that may evolve to severe enteropathy associated with T cell lymphoma, prognosis being poor. RCD is diagnosed in those over 50 years old, but may appear earlier in life too. Respecting the sex ratio of CD, RCD is also 2 – 3 times more prevalent in women. The incidence of RCD varies between 0.04 and 1.5%. The diagnosis of RCD II becomes more obvious when associated with severe malnutrition, enteropathy with protein loss, ulcerative jejunitis. The symptoms of RCD I are less severe, and the endoscopic and histological aspects follow closely the pattern of an active, uncomplicated CD. RCD I is difficult to distinguish from cases of slow response to diet or accidental exposure to gluten, as there are no definitive diagnostic criteria. Compared with RCD II, RCD I causes a milder form of malnutrition and ulcerative jejunitis, is less frequent and less severe (1, 11, 62, 63).

There are studies in which the authors tried to demonstrate whether the modulation of miRNAs is dependent on clinical symptoms, and they studied the expression profile changes of miRNAs from the duodenal mucosa of CD patients in relation to the specific clinical manifestations (64). The results are shown in the *Table 3*.

Celiac disease can be associated with various other diseases such as selective IgA deficiency, dermatitis herpetiformis, alopecia areata, vitiligo, psoriasis, type 1 diabetes mellitus, Hashimoto's thyroiditis, Addison's disease, Graves' disease, Sjogren's syndrome, scleroderma, systemic lupus erythematosus, dermatomyositis, rheumatoid arthritis, polymyositis, myasthenia gravis, Down's syndrome, Turner's syndrome, William's syndrome, autoimmune atrophic gastritis, primary biliary cholangitis, autoimmune hepatitis, primary sclerosing cholangitis, microscopic colitis, inflammatory bowel diseases, autoimmune hemolytic anemia, IgA nephropathy (Berger's disease), multiple sclerosis, sarcoidosis and idiopathic dilated cardiomyopathy (1, 41) (*Table 4*).

Patients with dyspepsia have a 1% prevalence of CD, and a 3 – 10% prevalence of type 1 DM. In patients with type 1 DM, genetic testing and following antibody monitoring are recommended. CD can be safely excluded in patients negative for HLA-DQ2 and HLA-DQ8 antigens. If family history is positive for CD, even if both markers are negative, the patients still require periodic reassessment (65). First-degree relatives and monozygotic twins have a higher risk of developing CD (11) (*Table 4*).

Some authors consider that patients with irritable bowel syndrome (IBS) have a four-fold increase in relative risk compared to controls from the general population (66) (*Table 4*).

Table 3. Up- and down-regulated microRNAs (miRNAs) in the duodenal mucosa of adult CD patients with different clinical manifestations.

Categories	Up-regulated miRNAs	Down-regulated miRNAs	Ref.
All CD cases	miR-24-2-5p, miR-2113, miR-523-3p, miR-182, miR-4300, miR-449a, miR-503, miR-486-5p, miR-551b-5p, miR-644, miR-504, miR-519d, miR-21-3p, miR-330, miR-500, miR-196a, miR-492, miR-21-5p, miR-491-3p, miR-642b-3p	miR-202, miR-412, miR-105, miR-135a, miR-659, miR-380-5p, miR-379, miR-618, miR-614, miR-194-5p, miR-512-3p, miR-600, miR-197, miR-215, miR-338-3p, miR-189, miR-299-5p, miR-323, miR-124a, miR-576, miR-31-5p, miR-631, miR-566, miR-409-5p, miR-451a, miR-616, miR-219, miR-192-5p	23, 64, 100, 101
Cases with CD-related anemia	miR-337-3p, miR-3681-5p, miR-550b-3p, miR-300, miR-3663-5p, miR-146a-5p, miR-2355-3p, miR-4300, miR-1290, miR-642a-p/miR-642b-5p, miR-593-3p, miR-1273e, miR-498, miR-490-3p, miR-302a-3p, miR-3611, miR-4324, miR-1270, miR-3183, miR-3135a, miR-3148, miR-1285-3p, miR-3654, miR-422a, miR-519d, miR-4303, miR-551b-5p, miR-24-2-5p, miR-618, miR-1304-5p, miR-4329, miR-146b-3p, miR-1299, miR-432-5p, miR-638, miR-920, miR-4268, miR-491-3p	miR-451a, miR-194-5p, miR-192-3p, miR-31-5p, miR-138-1-3p, miR-30b-5p, miR-193a-5p, miR-192-5p, miR-215, miR-664-5p	23, 64
Cases with CD on gluten-free diet	miR-3681-5p, miR-1285-3p, miR-551a, miR-422a	N.A.	23, 64

Abbreviations: CD, celiac disease; GFD, gluten free diet; N.A., not available.

DIAGNOSIS OF CELIAC DISEASE

The diagnosis of CD is confirmed if histological changes in the duodenal mucosa are combined with positive serological tests. Elevated antibody titers and negative biopsy are characteristic of potential celiac disease, and over time villous atrophy can develop. Recent studies show that in some categories of children and adult patients, the diagnosis of celiac disease without biopsy, only by detecting specific antibodies, can be very precise (63). In children with IgA tTG values ≥ 10 times the upper limit of normal, diagnosis of celiac disease can be established without biopsy, with the agreement of the family and provided that IgA EMA is positive in the second blood sample, regardless of the presence of symptoms or genetic testing (HLA-DQ2/DQ8). For those with IgA tTG values < 10 times the upper limit of normal, biopsies from the duodenum and duodenal bulb are required (67).

Serological tests

For the diagnosis of celiac disease, serological tests indicate the presence of IgA tTG and IgA EMA. In patients with normal IgA levels, IgA tTG testing has 95% sensitivity and specificity. IgA tTG is a safe and less expensive method of diagnosing and monitoring celiac disease. While IgA tTG has a higher sensitivity and IgA EMA has a higher specificity, simultaneous testing of the two antibodies reaches a sensitivity and specificity > 95%. Patients with IgA deficit have the alternative of IgG DGP or IgG tTG determination (11).

Genetic tests

Genetic tests include determination of HLA-DQ2 and HLA-DQ8 genotypes, with a negative predictive value of over 99%. HLA-DQ2/DQ8 testing is required in the following situations: in patients who started the gluten-free diet before being tested, in those with discordance between serology and histology, in risk groups (family members, patients with other autoimmune diseases or genetic diseases) (11).

Endocopic and histological diagnosis

Endoscopy evidences several types of CD markers: atrophy (with visible submucosal vessels), mosaic or micronodular apearance, fissures (grooves between folds), loss or flattening of folds and flattened Kerckring folds. These markers are usually described in the descending portion of the duodenum, less attention being paid to changes in the duodenal bulb (68-70).

Histological diagnosis requires 4 biopsy samples from the 2nd and 3rd portions of the duodenum and 1 – 2 samples from the duodenal bulb. Biopsies are assessed by Marsh-Oberhuber and Corazza-Villanacci histological criteria (11).

These criteria include hyperplasia of crypts, lymphocytic infiltrate and villous atrophy. In rare cases lesions can be found only in the jejunum. For complicated and not clear-cut cases enteroscopy might be considered (71). Lymphocytic infiltrate alone (above 25 IEL per 100 enterocytes) does not necessarily involve CD etiology, as it may also be found in other conditions such as food allergies, infections (*Helicobacter pylori*, *Giardia*), some drugs (NSAIDS, PPIs), autoimmune diseases (rheumatoid arthritis, Hashimoto's thyroiditis, Crohn's disease, systemic lupus erythematosus, multiple sclerosis, autoimmune enteropathy), common variable immunodeficiency, graft-versus-host disease, bacterial overgrowth, blind loop syndrome, microscopic colitis, irritable bowel syndrome, non-celiac gluten sensitivity (NCGS) (11, 65).

There are also cases of villous atrophy that should be considered for the differential diagnosis of celiac disease: collagenous sprue, autoimmune enteropathy, Crohn's disease, common variable immunodeficiency, infections (*Giardia*, *Tropheryma whippelii*, *Mycobacterium avium complex*, *HIV*), some drugs (olmesartan, losartan, colchicine, mycophenolate mofetil), nutritional deficiency, eosinophilic gastroenteritis, bacterial overgrowth, enteropathy-associated T cell lymphoma

Table 4. At-risk comorbidities of celiac disease.

Endocrine diseases	**Digestive diseases**	References
Type 1 diabetes mellitus Hashimoto's thyroiditis Addison's disease, Graves' disease Sjogren's syndrome	Autoimmune atrophic gastritis Primary biliary cholangitis Autoimmune hepatitis Primary sclerosing cholangitis Microscopic colitis Inflammatory bowel diseases Dyspepsia Irritable bowel syndrome	1, 11, 41, 65, 66
Dermatological diseases	**Rheumatological diseases**	
Dermatitis herpetiformis Alopecia areata Vitiligo Psoriasis	Scleroderma Systemic erythematosus lupus Dermatomyositis Rheumatoid arthritis Polymyositis	
Genetic diseases	**Immunological diseases**	
Down's syndrome Turner's syndrome William's syndrome	Selective IgA deficiency	
Hematological diseases	**Neurological diseases**	
Autoimmune hemolytic anemia	Multiple sclerosis Myasthenia gravis	
Nephrological diseases	**Heart disease**	
IgA nephropathy (Berger's disease)	Idiopathic dilated cardiomyopathy	
Pulmonary diseases	**Others**	
Sarcoidosis	First-degree relatives Monozygotic twins	

Abbreviations: CD, celiac disease; IgA, immunoglobulin A.

(EATL), graft-versus-host disease, amyloidosis, chemoradiotherapy and immunomodulatory therapy (anti-CTLA4 antibody) (11, 65).

Additional diagnostic tests

Other tests that can be performed for the diagnosis of celiac disease: video capsule endoscopy (VCE) (useful for detecting the complications of celiac disease); intestinal fatty acid binding protein (I-FABP) (helpful for detecting dietary non-adherence and unintentional gluten intake); intestinal permeability tests; radiological investigations (11).

Diagnostic methods for refractory celiac disease

1. Adherence to diet and initial diagnosis of celiac disease

In instances when CD does not respond appropriately to gluten-free diet (GFD), the degree of diet observance should be the primary concern. Specific antibody levels (tTG and/or EMA) should be measured. Low antibody levels do not exclude RCD when they persist in the context of progressive auto-inflammatory reactions independent of gluten ingestion. If CD-related genotypes are not found (HLA-DQ2.5 or HLA DQ8) and/or specific antibodies were negative at the time of diagnosis, then the CD diagnosis was wrong (72, 73).

2. Upper gastrointestinal endoscopy and histological assessment

Upper gastrointestinal endoscopy with duodenal mucosal biopsies is recommended. Endoscopic findings in RCD may resemble uncomplicated active CD, while ulcerative jejunitis suggests RCD II. The persistence of villous atrophy on endoscopic examination requires looking for other histological causes of atrophy. When these have been excluded, the diagnosis of RCD may be established. In order to classify these patients, detailed analyses of the bioptic samples are necessary (11, 62, 72).

3. Identification of aberrant intraepithelial lymphocytes

The small bowel normally contains intraepithelial lymphocytes (IEL). Their number increases in CD and RCD I. Phenotypically they belong to the T cell class, expressing the surface markers CD3+ and CD8+, and intracellular CD3 (iCD3), which constitute < 10% of IEL, but in RCD II they may represent over 90% of IEL. The presence of more than 20% of aberrant IELs is indicative of RCD II, and differentiates it from RCD I and villous atrophy of other causes (74). Evidence of clonal rearrangement of TCR is required to confirm the diagnosis of RCD II. Initial studies showed the presence of clonal rearrangement in surface and intracellular CD3, which is easier to evidence in bioptic samples. These aberrant cells can be identified by immunohistochemical analysis, flow cytometry, PCR (11).

4. Imaging

Abdominal CT (mesenteric lymph adenopathies, intestinal wall thickening, spleen atrophy), VCE (intestinal injuries), enteral MRI (intestinal injuries), double balloon enteroscopy (intestinal injuries), PET CT (invasive lymphoma) (75-77).

COMPLICATIONS OF CELIAC DISEASE

The major complication of CD is intestinal T-cell lymphoma, which may occur significantly more often than in a control population in patients with severe long-term disease forms or refractory to a gluten-free diet. However, it is also very rare among people with CD (4).

Patients with CD present a high risk of lung infections (78), mainly with pneumococcus, sepsis and non-Hodgkin lymphoma (4, 79). It seems that a higher risk of *Clostridium difficile* infection is found in patients with CD, albeit uncertainties remain (80, 81).

Hyposplenism may affect more than one third of adults with CD, while this is not a complication in children and youths. The incidence of hyposplenism relates to the time length of gluten exposure, and is further increased in cases of associated autoimmune diseases or premalignancies (82). Based on these associated factors, the spleen function should be tested in older patients at the time of diagnosis, in patients with autoimmune diseases or premalignant conditions, and in those with major infections or thromboembolism (83).

If patients develop collagenous enteritis or intestinal ulcers as complications, prognosis becomes severe. Also, the prevalence of intestinal carcinoma (and esophageal carcinoma according to some authors) is higher in patients with CD (2).

The metabolic consequences related to bone mineral deficits such as fractures or other demineralization syndromes are important. Celiac disease is associated with many neoplasias, among which small bowel cancers, adenocarcinoma, esophageal lymphoma and cancer. These diseases can be prevented by early diagnosis (65).

TREATMENT OF CELIAC DISEASE

The only efficient intervention for CD is a gluten-free diet, which removes the immune triggers. Most patients self-report an improvement after several weeks or months (84). In a subset of patients, histological regeneration of the mucosa requires about 2 years of sustained gluten-free diet (GFD). The clinical relevance of the delay in histological healing is not evident, but studies show that these patients are at higher risk for complicated forms of CD and osteoporosis. In some patients GFD brings about no clinical improvement, the most likely cause being accidental gluten ingestion or intestinal disturbances similar to CD (72, 85-87).

An analysis of the tolerated gluten amount supports that less than 10 mg of gluten per day have no histological effect, 100 mg lead to visible alterations, and 500 mg cause well defined mucosal damage. It has been inferred that a 30 mg daily gluten intake might be safe for the intestinal mucosa. At present, a safety range can be therefore established between 10 and 100 mg daily. A systematic review of 35 studies also suggests that in patients with CD the tolerated gluten amount varies, which means that a daily intake of < 10 mg is very unlikely to cause significant histological alterations (88, 89).

According to the Commission Implementing Regulation (EU) No 828/2014 of 30 July 2014, “very low gluten” (reduced presence of gluten in food) and “gluten-free” (absence of gluten in food) are used to indicate a gluten content not exceeding 100 mg/kg and 20 mg/kg, respectively.

Another aspect of therapy is substitution of nutritional deficiencies, especially by vitamins and minerals. Thus, in documented deficiencies, supplements of B group vitamins, mainly B1, B12, folic acid, vitamin D, vitamin K, calcium, iron, zinc and magnesium are indicated (90). The administration of vitamins is enteral whenever possible, and only in severe forms of malabsorption will it be parenteral. In severe forms short-term corticotherapy can be applied, which will allow GFD to show its benefits. Cyclosporine, azathioprine, infliximab, thioguanine, cladribine (2-chlorodeoxyadenosine), stem cell autologous

transplant, radiochemotherapy can be used as therapeutic alternatives (4, 62, 91).

There are also new therapies under study: oral enzyme supplements, larazotide acetate (which maintains intact intercellular junctions), irreversible transglutaminase 2 inhibitor, HLA-DQ2 blocking treatment, Nexvax2 vaccine (desensitization therapy) (11).

At present, there is no standardized treatment regimen for celiac disease, but identification of interleukin 15-mediated antiapoptotic pathways might generate new therapeutic methods. Also, the role of a nutritionist is essential in managing the gluten-free diet as much as possible and in learning correct eating habits (11).

FOLLOW-UP OF PATIENTS WITH CELIAC DISEASE

Follow-up of patients with CD should be performed at 3 – 6 months from diagnosis, and then, laboratory tests should be repeated every year. In case symptoms and nutrition deficiencies persist, adherence to a gluten-free diet should be checked, by assessing the serum markers of CD (92). A decrease of the reference values may be noted within several months from GFD onset (93). If serum markers do not diminish after one year of gluten restriction, it usually means that gluten contamination occurred. Repeat biopsy is not a routine practice for all patients. It is needed in those whose symptoms persist despite a strict gluten-free diet and when RCD is suspected. Repeat biopsy after 1 – 2 years from the start of GFD is indicated in patients over 40 years, in those who initially presented severe manifestations and in those who were serologically negative from the beginning (11).

Other necessary measures for the follow-up of these patients include: pneumococcal vaccination in those with hyposplenism, osteodensitometry in those at high risk of osteoporosis, and testing of family members (11).

There are studies confirming the relationship between oxidative stress and the degree of duodenal atrophy assessed by Marsh score. Autoantibodies against pancreatic secretory-granule membrane glycoprotein 2 (GP2), especially of IgA isotype, have been studied in celiac patients and they could be used as follow-up markers of mucosal inflammation (94, 95). Some studies suggested to monitor GFD by directly detecting gluten immunogenic peptides in biological samples. These can interact with the immune system of patients with CD to induce an autoimmune response against tTG and other antigens (96-98).

Celiac disease is a condition with polymorphic manifestations, digestive, systemic and organic, whose prevalence and incidence are constantly growing.

There is an increasing proportion of asymptomatic patients that belong to risk groups, diagnosed by screening tests: from the relatives of CD patients to those with type 1 DM, dermatitis herpetiformis, aphthous stomatitis, autoimmune thyroiditis, systemic lupus erythematosus, rheumatoid arthritis, *etc*. Delay of diagnosis increases the mortality rate by malignant complications. The delay is mostly seen in patients with atypical manifestations or totally without symptoms, hence the importance of screening programs, at least in risk population groups.

Concerning celiac disease, elevated miR-449a levels were found to target and reduce the Notch1 pathway *in vitro*. This could be constitutively modified in the celiac small intestine and could lead to increased proliferation and diminished differentiation of intestinal cells, resulting in an increased proportion of the goblet cell type (99). Another study investigated the expression of miRNAs in duodenal biopsies in a sample of untreated CD patients of adult age (either typical or atypical presentation), a group of treated patients (with or without remission of mucosal damage) and control subjects without CD. Dysregulation of some miRNAs (miR-192-3p, miR-31-5p, miR-551a, miR-194-5p, miR-638, miR-551b-5p and miR-1290) was mentioned in CD patients compared to the control group. These miRNAs were subsequently examined in duodenal fibroblasts from CD patients and incubated with gliadin peptides (13- and 33-mer), demonstrating that the latter were regulators of matrix remodeling. MiRNA cluster miR-192/194, implicated in matrix remodeling, was modified in CD and related to clinical manifestations (64).

A list of relevant miRNAs and the modulating genes was sampled in duodenal bioptic material of pediatric CD patients. MiR-31-5p and miR338-3p were also reduced in patients and the genes they control, FOXP3 and RUNX1, respectively, were induced. The two above genes are involved in regulating the function of T lymphocytes. In the end, an increased expression of miR-21-5p was established in CD patients with its putative target STAT3. In a pediatric CD population, the regulation of autophagy might also be relevant, as indicated by the reduced expression of miR-17 and miR-30a (100, 101).

A study has shown that the expression pattern of miRNAs is different between the mucosa of CD patients and that of normal controls, and that the low levels of certain miRNAs led to up-regulation of a series of proteins involved in innate or adaptive immunity. These findings are induced by exposure to gluten, but only in CD patient biopsies, pointing to the possible involvement of miRNAs in the response to gluten and subsequent immune regulation (102).

Finally, the complexity of this disease and its clinical management could lead to new interesting perspectives in CD diagnosis and therapy research.

Abbreviations: CD, celiac disease; CD4, cluster of differentiation 4; CT, computed tomography; Cu, copper; DC, differentiation class; DGP, deamidated antigliadin antibodies; DM, diabetes mellitus; EMA, anti-endomisium antibodies; Fe, iron; GFD, gluten free diet; GWAS or GWA study, genome-wide association study; HLA, human leukocyte antigen; IEL, intraepithelial lymphocytes; IFN-γ, interferon gamma; IgA, immunoglobulin A; IgG, immunoglobulin G; IL, interleukin; MRI, magnetic resonance imaging; mRNA, messenger RNA; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NK cell, natural killer cell; NSAIDS, nonsteroidal antiinflammatory drugs; PCR, polymerase chain reaction; PET, positron emission tomography; PPIs, proton pomp inhibitors; RNA, ribonucleic acid; SNP, single-nucleotide polymorphism; TCR, T-cell receptor; Th, T helper; TNF-α, tumor necrosis factor alpha; tTG antibodies, anti-transglutaminase antibodies; Zn, zinc.

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