

CELIAC DISEASE: ROLE OF GENETICS AND IMMUNITY AND UPDATE ON NOVEL STRATEGIES FOR TREATMENT

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*Celiac disease (CD) is one of the most common inflammatory diseases of the small intestine which causes abdominal pain, diarrhoea, malabsorption, weight loss, anorexia, and iron deficiency anaemia in humans. It is a human leukocyte antigen (HLA)-linked disorder that is triggered by the gluten and gliadin proteins from wheat and related cereals. The presence of other genetic factors such as HLA-DQ2 and HLA-DQ8 have also been identified for the generation of circulating autoantibodies to the enzyme transglutaminase (TG2). The TG2 enzyme deamidates the gluten peptides and increases their affinity for the HLA-DQ2 or HLA-DQ8, which in turn cause a more vigorous activation of CD4⁺ T-helper 1 (Th1) cells and trigger the immune response, and such immune cascade eventually leads to intestinal membrane damage and malabsorption. Generally, CD is managed by life-long gluten-free diet. However, strict adherence to a gluten-free diet is difficult and is not always effective. Several pharmacological agents and alternative therapies for treating CD are currently under development and are in clinical trials, The purpose of this review is to highlight the complex involvement of genetics and immunity in CD and to focus on the novel strategies being used for developing adjunct and alternative therapies for the treatment of CD. **Biomed Rev 2014; 25: 45-58***

Key words: HLA-DQ2, HLA-DQ8, gluten, gliadin, intraepithelial lymphocytes, genetic predisposition

INTRODUCTION

Celiac disease (CD) is a one of the most common inflammatory disease of the small intestine that is triggered in genetically predisposed individuals by gluten and gliadin proteins present in wheat, barley, and rye. CD manifests in various degrees ranging from intraepithelial lymphocytosis to severe

subepithelial mononuclear cell infiltration resulting in total villous atrophy coupled with crypt hyperplasia. Through autoantibody and biopsy screening, the incidence of CD in the United States and other Western and Middle Eastern countries was found to be between 1:70 and 1:200 in human popula-

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tion (1-8). Celiac disease is often found in conjugation with other autoimmune disorders such as type-1 diabetes mellitus, autoimmune thyroiditis and dermatitis, herpetiformis and autoimmune alopecia (9). In addition, patients with long-standing undetected or untreated symptomatic CD are likely to be at a higher risk of developing the enteropathy-associated with T-cell lymphoma, small bowel adenocarcinoma and other cancers of the gastrointestinal tract (10-13). Generally, CD is linked with major histocompatibility complex class II genes and the alleles encoding the human leukocyte antigen molecules HLA-DQ2 and HLA-DQ8 and almost all patients with CD carry these HLA alleles (14). However, CD has a multifactorial inheritance. Therefore, its occurrence not only depends on specific mutations of a single gene but also can be caused by a combination of environmental factors and variations in multiple genes (15, 16). Also the viral infections altering intestinal permeability, gut microbiota, breast-fed infants and timing of gluten introduction in infant's diet has been implicated in the development of CD (17-19).

While some environmental factors play a role in causing CD, gluten is one of the important players. Gluten-containing cereals have a high number of repetitive glutamine- and proline-rich sequences, thus making them highly resistant to proteolytic degradation by gastric, pancreatic, and intestinal brush-border enzymes, even in case of healthy individuals (20,21). Such proteolytic resistance results in the persistence of relatively large peptides in the gastrointestinal tract, which activate the small-bowel mucosal immune system, thereby leading to the development of CD in genetically predisposed individuals. Under normal physiological conditions, the intestinal epithelium is fairly impermeable to long peptides. However, in undiagnosed or untreated CD, the epithelial barrier function is compromised and gliadin peptides gain access across the epithelial layer and activate the immune system (22).

Currently, the available treatment of CD is a lifelong strict gluten-free diet, which helps to relieve symptoms in most cases and effectively prevent the potential malabsorption problems (6, 23-29). However, it is difficult to maintain the gluten-free diet schedule and such practice can lead to social isolation, since modern diets are heavily based on gluten containing products. The lifelong strict gluten-free diet can also have an adverse impact on the quality of life of patients (30, 31). There are many instances where the gluten-free diet has been contaminated with wheat flour (32, 33). Owing to all these disadvantages and ineffectiveness of gluten-free diet in some cases, there is an increased need of alternative new therapeutic

approaches. These novel therapies are being developed for the management of CD, based on the enhanced understanding of the pathogenic mechanisms that underline this disease.

ROLE OF GENETICS AND IMMUNITY IN CELIAC DISEASE

HLA class I and II genes map on the short arm of chromosome 6 (6p21.3) and codes for the cell surface antigen presenting proteins and thus play a major role in immunity (34, 35). HLA class I heterodimers are constituted of alpha-heavy chain that encodes the HLA-A, B and C loci and by small beta 2-microglobulin molecule whose gene maps on chromosome 15. HLA class II heterodimers that consists of alpha and beta chains are specified by genes in the HLA-D region that comprehends HLA-DP (DPA1 and DPB1), DQ (DQA1 and DQB1) and DR (DRA1 and DRB1) genes (36). Among the genetic markers, it is the HLA gene that seems to make greatest contribution to CD (37). The presence of the genes that are responsible for coding DQ2 and DQ8 molecules of HLA complex class II explains up to 40% of the occurrence of CD in European population (38). The association between DQ2 and DQ8 with CD is due to the high affinity of the DQ2 molecule of HLA towards the peptides- derived from gluten, which present them to the T lymphocytes (39). According to a multicenter study carried out in Europe, it was observed that the HLA DQ2 was present in about 86 to 93% of CD patients, while around 3 to 8% of these patients have DQ8 without DQ2 (40). Interestingly enough, these molecular markers are also carried by many individuals without CD, for example, in 40 to 65% of first degree relatives of CD patients and 18 to 30% of the normal population (41-43). Thus, CD has a multifactorial inheritance factors, and does not depend on the mutations of a single gene but it is caused by a combination of environmental factors and variations in multiple genes. In recent years, genome-wide association studies (GWAS) have identified many non-HLA genes associated with an increased risk of CD as indicated in Table 1, such as those coding for cytokines, chemokines and their receptors, cell adhesion molecules, T- and B-cell activators.

The non-HLA genetic contribution to celiac disease is only 15% and these polymorphisms are not considered in the calculation of the genetic risk (36). The primary HLA-DQ association in CD was initially confirmed by experiments on CD4⁺ T lymphocytes which were isolated from intestinal biopsies of patients who were able to recognize gluten peptides which were presented by DQ2.5/ DQ8 positive antigen presenting cells (60-63). The DQ2.5/DQ8 displays the highest affinity for the negatively charged amino-acids derived from the TG2 mediated deamidation (i.e. conversion of glutamine to glutamic

Table 1. *Non-HLA loci and genes of celiac disease susceptibility*

Loci identified	Origin of the cohort	Candidate genes	References
CELIAC 2 5q31-q33	Italy, Finland, Scandinavia, Europe	Unknown	44-47
CELIAC 3 2q33 CELIAC 4 19p13.1	France, The Netherlands, Sweden, Norway Netherland	CTLA4 Myosin IXB	48-50 51
CELIAC 5 15q11.q13	Finland	Unknown	52
CELIAC 6 4q27	United Kingdom, Netherland, Ireland, Italy, Scandinavia, United States	KIAA1109 TENR (AD AD 1) IL2 IL21	53-57
CELIAC 7 1q31	United States, United Kingdom, Italy, Ireland, Netherland	RGS1	53,54,56,57
CELIAC 8 2q11-q12	United Kingdom, Ireland, Netherland	IL18RAP IL18R1	53,54,58
CELIAC 9 3p21	United Kingdom, Ireland, Spain, Netherland	CCR1 CCR2 CCRL2 CCR3 CCR5 XCR1	53,54,59
CELIAC 10 3q25-q26	United Kingdom, United States, Ireland, Italy, Netherland	IL12A	53,54,56,57
CELIAC 11 3Q28	United Kingdom, United States, Ireland, Italy, Netherland	LPP	53,54,56,57
CELIAC 12 6q25.3	United Kingdom, Ireland, Italy, Netherland	TAGAP	53,54,57
CELIAC 13 12q24	United Kingdom, United States, Ireland, Italy, Netherland	SH2B	53,54,56,57

acid amino-acid) (64). This degree of affinity is dependent on the degree of interaction that occurs in the central region of nine amino-acid residues whose lateral positions are anchored to the positions P₁, P₄, P₆, P₇, and P₉ into DQ-binding regions. Specifically, it is the DQ2.5 heterodimers that bind peptides with negatively charged side chains at P₄, P₆ and P₇ positions while DQ8 molecules show only a preferential binding for the negatively charged residues at P₁ and P₉ (65). The role played by specific DQ heterodimers is being studied so as to understand the cause of the gluten-specific T-cell response in patients carrying other DQ molecules (not DQ2.5 or DQ8) (63, 66-68). Thus, it appears that the genetic predisposition to CD depends on one gene with a large effect (HLA-DQ2/DQ8) on the adaptive immune response to gluten peptides and many other genes influencing different aspects of innate and adaptive immune reactions, intestinal permeability, and general predisposition to autoimmunity.

HLA MOLECULAR TYPING

So far, only HLA-DQA1 and HLA-DQB1 loci can be used in the clinical practice of CD (69). By itself, HLA test does not have a diagnostic significance but is mainly considered for its negative predictive value since CD is highly unlikely to occur when DQ predisposing alleles are absent; while a positive result only means a genetic predisposition for celiac autoimmunity (36). Although many individuals carry DQ2.5/DQ8 molecules but they do not depict the disease and only some will develop gluten intolerance. Thus, it is vital that future clinical investigations should address to identify new markers, which can improve prediction of the disease in people who carry the same HLA at-risk alleles. Sequence Specific Primers-PCR, Reverse Dot Blot analysis and Real Time PCR have been used for HLA molecular typing, and recently, different commercial kits have been developed to specifically genotype CD-associated DQA1/DQB1 alleles (70, 71). Thus, HLA typing allows to define the CD risk gradient associated with each particular HLA-DQ status and DQB1*02 homozygosity/heterozygosity (72, 73). HLA typing is routinely suggested by clinicians in dubious CD case, i.e., uncertain or discrepant serology and or biopsy (74, 75). It is useful in screening first degree relatives due to the high prevalence of CD in first degree relatives.

Almost all the patients with CD develop the immunoglobulin (Ig) A autoantibodies to TG2 enzyme. The TG2 is responsible for deamidation of glutamine to negatively charged glutamic acid residues (76, 77). Due to their high content in glutamine and neighbouring proline and hydrophobic amino

acid residues, gluten proteins, are substrates for TG2 (76, 78). When deamidated, the affinity of the negatively charged gluten peptides to HLA-DQ2 or HLA-DQ8 increases which results in a vigorous T-cell activation. While the adaptive immune response to gluten is well established, the gluten proteins can elicit an innate immune response in professional antigen-presenting cells that activates predominantly the intraepithelial lymphocytes (IEL) but also the intestinal epithelial cells (79-83). This innate immune response is an immediate reaction that appears to favour the development of adaptive immunity to gluten in patients who carry HLA-DQ2 or HLA-DQ8 (80). The innate immune activation of IELs by gluten induces the expression of the nonclassical class I molecule MICA on the intestinal epithelium which serves as a ligand for the heterodimeric NKG2D receptors.

It has been observed that epithelial MICA and up-regulated epithelial production of interleukin (IL)-15 leads to the activation of NKG2D on IELs (84). NKG2D also links the innate and adaptive immunity, because it triggers both antigen-specific lymphocyte mediated cytotoxicity and induces a direct cytolytic function independent of T-cell receptor (TCR) specificity in effector CD8 T cells (85). The central role of IL-15 in the activation of innate and adaptive immunity in CD has been confirmed by several investigators (86-90), along with an increased expression of IL-15 receptor and a lower threshold for activation on IELs (88). Recently, it was found that IL-21, which is produced by CD4⁺ Th1 T-cells, has emerged as an additional driving force to the innate immunity that often acts in concert with IL-15 (91). It is imperative to know the pathogenesis of CD because it is one of the important causes of malabsorption in humans. The data analyses of a clinical study using endoscopic and histological features of 94 consecutive patients over 12 years indicated that CD is the most common cause of malabsorption followed by tropical sprue (92). According to this study, 85.7% of the patients with tropical sprue had normal duodenal folds, whereas 82% of the patients with CD had abnormal duodenal folds. Also, the scalloping of folds as well as both scalloping and attenuation of the duodenal folds was more frequently present in patients with CD. On histological examination, it was observed that all the patients with CD had Marsh grade 3 villous atrophy, while only 11 patients with tropical sprue had Marsh grade 3 villous atrophy. Crypt hyperplasia was greater in CD than that of tropical sprue.

While increase in IELs were noted in all the biopsies, grade 3 IEL infiltrates were seen only in CD. The extent of epithelial cell damage in the biopsies was focal in 85.7% of the patients

with tropical sprue, the extent of epithelial damage was mostly diffuse in those with CD. In addition, the normal tall columnar epithelium shape was maintained in most patients with tropical sprue, but changed from normal tall columnar epithelium to cuboidal cells in 40.9% of patients with CD (92). The marked histopathological differences observed between CD and tropical sprue patients should be taken into consideration while examining the duodenal biopsy samples or doing the endoscopic examination of the GI tract.

Celiac Disease has numerous symptoms as indicated in Table 2. These symptoms vary with different people. These differences make the diagnosis of the disease difficult.

Table 2. Symptoms of celiac disease*

Gastrointestinal symptoms	Non-gastrointestinal symptoms
Diarrhoea	Iron deficiency anaemia
Weight loss	Chronic pain
Abdominal pain	Migraine
Anorexia	Joint pain/ inflammation
Abdominal distension	Attention deficit disorder
Lactose intolerance	Depression
Abdominal distention	Epilepsy
Irritability	Osteoporosis/ Osteopenia
	Infertility/ Recurrent fetal loss
	Autoimmune disorders
	Delayed puberty

*From (93)

THE INTERVENTION OF NOVEL THERAPIES

As mentioned earlier, an efficient therapy for the patients suffering from CD is ideally the adherence to a strict gluten-free diet, which is difficult to maintain and comply with. Such restriction may not only interfere with many social activities, but may also limit the enjoyment of food varieties. Furthermore, patients who have a high level of gluten sensitivity are likely to be adversely affected by even small amounts of gluten in their foods which may be declared gluten-free. Therefore,

exploration of alternative therapies or adjunctive treatments becomes necessary for the CD patients.

WHEAT VARIANTS AND GENETIC MODIFICATION

Wheat strains with low immunogenicity, i.e., decreased number of immunogenic T-cell epitopes can be selected from the already existing varieties or from the genetically modified wheat. By hybridization between tetraploid *Triticum turgidum* and the diploid *Triticum tauschii*, the hexaploid *Triticum aestivum* was generated. The tetraploid *Triticum turgidum* likely originated from the diploid *Triticum monococcum* and *Triticum speltoides* (94). The duodenal biopsy specimens from patients with celiac disease showed that ingestion of tetraploid wheat gluten caused reduced toxicity as opposed to the intake of hexaploid wheat (95). Similarly two other wheat varieties, one low in α - and β -gliadins and another low in α -, β -, γ - and ω -gliadins also showed decreased toxicity in duodenal biopsy specimens (96).

With the utilization of gluten-specific T-cell clones from duodenal biopsy specimens of CD patients and the identification of key immunogenic T-cell epitopes, including the antibodies directed to some of these epitopes, provided reproducible results for the characterization of less toxic wheat species. *In silico* approach was applied to analyze 230 α -gliadin sequences derived from ancestral haplotypes for the presence of T-cell stimulatory epitopes that bind to HLA-DQ2/8. It was observed that all major immunogenic peptides are present in the DD genotype, except for α -9 sequences in the AA genotype. The gluten digest from *T. tauschii* which contains the α -gliadin sequences that is encoded by the DD genome elicited the strongest T-cell response as compared to the gluten derived from the AA and BB genome species that lack these sequences were dampened (97, 98).

Genetic deletion of certain gliadin genes was analyzed in *T. aestivum*, which lacks one locus containing gluten genes. This was carried out by *in silico* analysis based on the known DNA sequences and by Western blotting with epitope specific antibodies (99). The results revealed that the complete deletion of the α -gliadin locus on chromosome 6 led to a decrease in total T-cell stimulatory epitopes, however, it also impaired the baking properties of this wheat variety.

PRETREATMENT OF FLOURS AND GERMINATION OF WHEAT

Certain lactobacilli added to sourdough for fermentation are able to proteolyze the proline- or glutamine-rich gluten peptides and thus decrease the immunotoxicity (100-102). A pilot

study done in 17 CD patients suggested that the sourdough bread was well tolerated. Since the patients were challenged only for 2 days, hence it was difficult to draw any definitive conclusions from this very short period study (103). In a similar manner, intrinsic proteases are produced during germination of wheat, i.e., when the amino acids from the gluten storage proteins are being used by the growing plant. The germination process of wheat can degrade the immunogenic T-cell epitopes, and thus create nontoxic cereal product for patients suffering from CD (104). Another alternative would be to inactivate immunogenic gluten epitopes by exploiting the substrate specificity of TG2 that generates more potent gluten peptides via deamidation (105).

The treatment of wheat with low molecular weight microbial TG, derived from *Streptomyces moboraensis*, reduced the stimulatory effect of the flour on gluten reactive T-cells. Additionally, treatment of TG with these microbes improves loaf volume and crumb texture of breads (106). Nevertheless, caution is necessary since the treatment of flour with the microbial TG increased rather than decreasing the stimulation of gliadin-specific T-cells (107, 108). Thus, the future studies should focus on the development of cereals that are devoid of immunogenic epitopes along with conserving their baking qualities.

ORAL ENZYME THERAPY

Usually the complex proteins that reach the intestinal lumen are digested by gastric pepsin and pancreatic proteases and further degraded by the brush border enzymes into the simpler amino acids, dipeptides or tripeptides that are transported across the epithelial layer. The large quantity of proline residues in immunodominant gliadin peptides which make them highly resistant to the human digestive proteases (109-111). Hence, in order to avoid those peptides from reaching the lamina propria, prolyl-endopeptidases (PEP) which are expressed in many microorganisms were used to cleave the immunodominant proline rich regions (112-116). A study was performed using PEP obtained from *Flavobacterium meningosepticum* admixed to a daily drink with 5 grams of gluten over 2 weeks, and it could prevent fat malabsorption and symptoms in some patients with previously diet controlled CD (117). However, the potency of the enzyme was not considered sufficient enough to draw any clear conclusions. The PEP from *Aspergillus niger*, which has a higher specific activity than PEP from *Flavobacterium meningosepticum* to inactivate the immunodominant gluten peptides may be tried (118, 119).

INHIBITION OF INTESTINAL PERMEABILITY

In healthy individuals, the tight junctions between the epithelial cells protect and control the exposure of the submucosal gastrointestinal tissues to the macromolecules which may be toxic. However, in patients with CD there is an increased intestinal permeability due to the opening of the tight junctions (*zonulae occludentes*), causing an increased influx of the gluten peptides into the subepithelial *lamina propria* and the T-cell response to gluten is triggered. Previous studies identified that the human protein zonulin, a precursor of prehaptoglobin-2, acts as a regulator of epithelial permeability and is highly expressed in CD and is likely a contributor to its pathology (120,121). This protein is similar in effect to that of *zonula occludens toxin* (ZOT) expressed by *Vibrio cholerae*, which impairs epithelial tight junctions integrity (122). An octapeptide derived from ZOT (AT-1001) antagonises zonulin action *via* receptor blockade and thus protects tight junctional integrity (123). A pilot study was performed using AT-1001 in 14 patients with CD in remission and 7 controls who were challenged with a single dose of gluten for three consecutive days, and it was observed that the intestinal permeability remained intact (124). It appears that a complementary therapeutic approach and its combination with other treatments could be more effective for treating CD patients.

TRANSGLUTAMINASE-2 INHIBITORS

The use of transglutaminase-2 (TG-2) inhibitors is also one of the novel therapy because inhibiting the deamidation of the gliadin peptide decreases its affinity to HLA-DQ2 and HLA-DQ8 and consecutively decreases the T-cell stimulation. Many inhibitors that target the transglutaminase cross-linking activity have been developed and tested *in vitro* (125-128). There are several competitive inhibitors of TG-2 (putrescine, spermidine, histamine, monodansyl cadaverin, cadaverine, 5-pentylamine, fluoresceine, cystamine and cysteamine) (129); reversible inhibitors (mainly guanoside triphosphate analogues) (130) and irreversible inhibitors (iodoacetamide, 3-halo-4,5-dihydroisoxazoles, carbobenzyloxy-L-glutaminy glycine derivatives, 6-diazo-5-oxo-norleucine, 2-[(2-oxopropyl)thio]imidazolium derivatives) (131,132). Cystamine and 2-[(2-oxopropyl)thio]imidazolium inhibitors (L682777 or R283) have also been tested *ex vivo* in cultures of small intestinal biopsy specimens of patients with CD where there was a decrease in the T-cell stimulatory activity of gliadin peptides (133,134). However, this therapeutic approach is risky, since transglutaminase plays many important roles in

the body and the inhibitory agents have to be designed so that they specifically remain in the intestine and do not cross to the systemic circulation.

HLA-DQ2 INHIBITORS

Adaptive immunity in CD is activated due to the binding of the gliadin peptides onto the HLA-DQ2, thus blocking the DQ2 as an attractive target to prevent the immune activation. Modification of α - gliadin so as to make it a partial agonist, significantly inhibited the IFN- γ production as compared to the patients with CD having the unmodified peptide (135). Furthermore, replacement of leucine L11 and L18 residues in the α -gliadin with sterically bulky groups retained the DQ2 affinity but decreased the T-cell recognition (136). Overall, if the designed inhibitors act locally in the gut and specific for gluten, it could constitute a potential therapeutic approach.

ANTI-INFLAMMATORY COMPOUNDS

One characteristic feature of CD is persistent inflammation of the intestinal tract. Treatment with corticosteroids and immunosuppressive agents have been considered. In a cohort study of 30 people, a combination of gluten-free diet and corticosteroids was found to be effective in treating the symptoms and healing of mucosal injury (137). Budesonide, a locally acting corticosteroid with minimal side effects when used in addition with gluten-free diet significantly improved the malabsorption symptoms (138). There also has been a report of mesalamine to treat the refractory sprue patients (139).

IL-15 ANTAGONISTS

An increased expression of IL-15 is responsible for the development of refractory CD, since it induces the secretion of epithelial MICA that binds to NKG2D receptor located on the surface of intraepithelial lymphocytes. A study on transgenic mouse models with overexpression of IL-15 and consequent development of autoimmune enteropathy indicates that blocking antibody against IL-15 was capable of efficiently reversing the intestinal damage (140, 141). IL-15 blocking antibodies were able to decrease the number of intraepithelial lymphocytes that accumulated in the intestinal epithelium of human IL-15 transgenic mouse models (142).

CXCL10 AND CXCR3

It was found that the CXCL10 which is another T-cell recruiting chemokine that elicits its effects by binding to the receptor CXCR3 expressed by T-lymphocyte showed an increased

expression in response to the gliadin stimulation of monocytes (143). These findings support the role of T cell-recruiting chemokines as a future therapeutic target not only for CD but also for gluten sensitivity in general (144).

CONCLUSIONS

In humans, CD is a leukocyte antigen (HLA)-linked disorder that is triggered by the gluten and gliadin proteins present in wheat, rye and barley. It appears that early humans didn't eat cereal grains, so people didn't evolve the ability to break down and digest gluten. Thus, in some subjects the body treats gluten as a toxin that triggers gut reactions. Globally, increased number of patients are diagnosed with CD. This chronic inflammatory disease of the small intestine causes abdominal pain, diarrhoea, malabsorption, weight loss, anorexia, and iron deficiency anaemia.

Due to better understanding of the underlying pathology, there has been a development of adjunctive and even alternative therapeutic approaches that help effectively to manage or even cure this condition. Although several pharmacological agents for treating CD are currently under development and are in clinical trials, gluten-free diet still remains the only treatment option for these patients, who regardless of the social aspect lack any significant or potentially life threatening side effects. Therefore, in addition to therapeutic efficacy for reducing both CD symptoms and histological intestinal damage, the ideal alternative therapy to lifelong gluten withdrawal will need development of safe and efficacious products. This encouraging trend could gain momentum in the coming years.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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