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REVIEW

The Gluten Gene: Unlocking the Understanding of Gluten Sensitivity and Intolerance

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Abstract: Wheat flour is one of the most important food ingredients containing several essential nutrients including proteins. Gluten is one of the major protein components of wheat consisted of glutenin (encoded on chromosome 1) and gliadin (encoded on chromosome 1 and 6) and there are around hundred genes encoding it in wheat. Gluten proteins have the ability of eliciting the pathogenic immune responses and hypersensitivity reactions in susceptible individuals called "gluten-related disorders (GRDs)", which include celiac disease (CD), wheat allergy (WA), and non-celiac gluten sensitivity (NCGS). Currently removing gluten from the diet is the only effective treatment for mentioned GRDs and studies for the appropriate and alternative therapeutic approaches are ongoing. Accordingly, several genetic studies have focused on breeding wheat with low immunological properties through gene editing methods. The present review considers genetic characteristics of gluten protein components, focusing on their role in the incidence of gluten-related diseases, and genetic modifications conducted to produce wheat with less immunological properties.

Keywords: gliadin, glutenin, genetic loci, wheat allergy, celiac disease, non-celiac gluten sensitivity

Introduction

Gluten-containing grains are essential food ingredients, consumed in most parts of the world.[1,2] Owing to their importance and thanks to their ability to grow in different climatic areas, wheat cereals were among the first crops to be cultivated (established in the "Fertile Crescent", such as modern Turkey, Iraq and Iran) and their importance has increased significantly over time.[3–6] The complex genome of Triticum aestivum L. is arranged into three subgenomes, A, B, and D, each contains seven pairs of chromosomes.[7,8] However, the ability of wheat to adapt to different eco-climatic conditions and deliberate breeding for specific traits have led to the emergence of varieties with different characteristics.[8] Accordingly, evidence showed that changes in the environmental conditions (such as temperature, water, and fertilizer situations) could influence the expression of gluten genes in wheat.[9]

Wheat (Triticum aestivum L. $2n = 6 \times = 42$) flour is composed of starch (~70–-75%: main component), proteins (~10–15%), lipids (~2%), minerals (~2%), that convey substantial nutritional benefits to humans.[10] Gluten is one of the major protein components of wheat (~80% of the total proteins), which is specifically expressed in the developing grains and provides a source of nitrogen for germination and seedling growth.[4,11–13] Gluten is composed of storage proteins including glutenin and gliadin and is the term applied to the viscoelastic matrix formed when

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these proteins are mixed with water. There are around hundred of genes encoding gluten proteins in wheat.[14–16] In general, wheat proteins are divided into water/salt-soluble and insoluble categories, of which gliadins and glutenins are insoluble components.[17] As gliadins influence the extensibility and viscous nature and glutenins are responsible for the elasticity and strength of dough, gluten is known as the main factor in determining the quality of the baked products and processed foods' texture and flavor.[18–20] Due to the high amount of proline (P) and glutamine (Q) residues in gluten, T.B. Osborne, the father of plant protein chemistry, called it "prolamine".[4,11,21] Accordingly, gliadin and glutenin are known as prolamin I and II, respectively.[22] Prolamines in other cereals include secalin in rye, hordein in barley, avenins in oats, zeins in corn, but the medical use of the term "gluten" has evolved to include only those prolamines implicated in human disease.[23]

Despite the numerous benefits of wheat, gluten proteins have the ability of eliciting the pathogenic immune responses and hypersensitivity reactions in susceptible individuals known as "gluten-related disorders (GRDs)".[4,23,24] GRDs encompasses three major types of diseases: celiac disease (CD), wheat allergy (WA) and non-celiac gluten sensitivity (NCGS) that affect around 1–7% of people worldwide.[4,25–27] These are, biologically, different diseases with distinct immune, allergic, and possibly non-immune etiologies for which gluten or wheat flour is a common triggering factor that will be discussed in detail below.[28]

Nevertheless, numerous studies, based on findings showing that avenins alone do not induce immune responses in most CD patients and symptomatically tolerated by them, point to the safety of adding oats to their diet.[29–32] In this regard, however, Hardy et al[33] in their in vivo study on 73 biopsy-confirmed HLA-DQ2.5+ CD patients showed that the ingestion of oats (100 g/day) for 3 days mobilizes polyclonal avenin-specific T-cells in blood in fewer than 10% of studied patients. Half of the patients had at least one digestive symptom during this challenge, which was due to a high daily intake of oats (100 g) and a high amount of fiber in them. Moreover, they reported that these T-cells were cross-reactive against avenin and hordein, and oral challenge with barley (and not wheat or rye) could stimulate these T-cells more efficiently than oats. They concluded that daily consumption of up to 100 g uncontaminated oats is insufficient to cause clinical relapse in CD patients.[33] A plausible explanation for oats having low immune-toxicity is their low proline content and lack of proteolytically resistant peptides with more than 10 amino acid residues.[33,34]

This review aims to provide a thorough overview of genetic characteristics of gluten protein components, their role in the incidence of various gluten-associated diseases, and genetic modifications that could reduce the immunogenic properties of gluten and lead to wheat improvement.

Method

In general, searches are developed in PubMed, Google Scholar, MEDLINE, and SCOPUS databases from September 1987 to September 2020. The following terms, alone or in combination, were searched: "gluten content", "gliadin chromosomal locations", "glutenin chromosomal locations", "celiac immunogenic peptides", "wheat allergy immunogenic peptides", "immunogenic peptides and non-celiac gluten sensitivity", "toxic gluten", and "gluten genetic manipulations".

Gliadin Components and Genetic Characteristics

Gliadin is a combination of monomeric proteins that makes up about 30% of total flour proteins.[4,35,36] Polyacrylamide gel electrophoresis at acidic pH (pH = 3.1) shows four major groups called α- (25–35 kDa), β- (30–35 kDa), γ- (35–40 kDa), and ω- (55–75 kDa) gliadins.[2,4,8,37] As α- and β-gliadins have several similarities in their structure and number of amino acid residues, they are usually grouped and collectively named as α-gliadins.[8,38] α/β- and γ-subunits are considered to be the major components of gliadins, and ω-gliadin is lower.[39] In addition, the ω- gliadin differs in amino acid composition from those of the α- and γ-gliadins.[4,11] Moreover, according to the Shewry classification based on the presence of sulfur-containing amino acids, gliadin subunits are divided into S-rich (α/β- and γ-) and S-poor (ω-) gliadins.[11]

The gliadin is encoded by multigene families.[40] Different reports on the chromosomal location showed that gliadin encoding genes are found on the short arm of the homoeologous group 1 (*Gli-A1, -B1* and *-D1* loci) and 6 (*Gli-A2, -B2* and *-D2* loci) chromosomes.[2] Each of these loci contains multiple alleles and so far, more than 30 allelic variants have been identified for some *Gli* loci.[2,37] Gli-1 genes encode γ - and ω-gliadins and Gli-2 genes encode the α-/β- and some of the γ-gliadins (Figure 1).[34] There are also

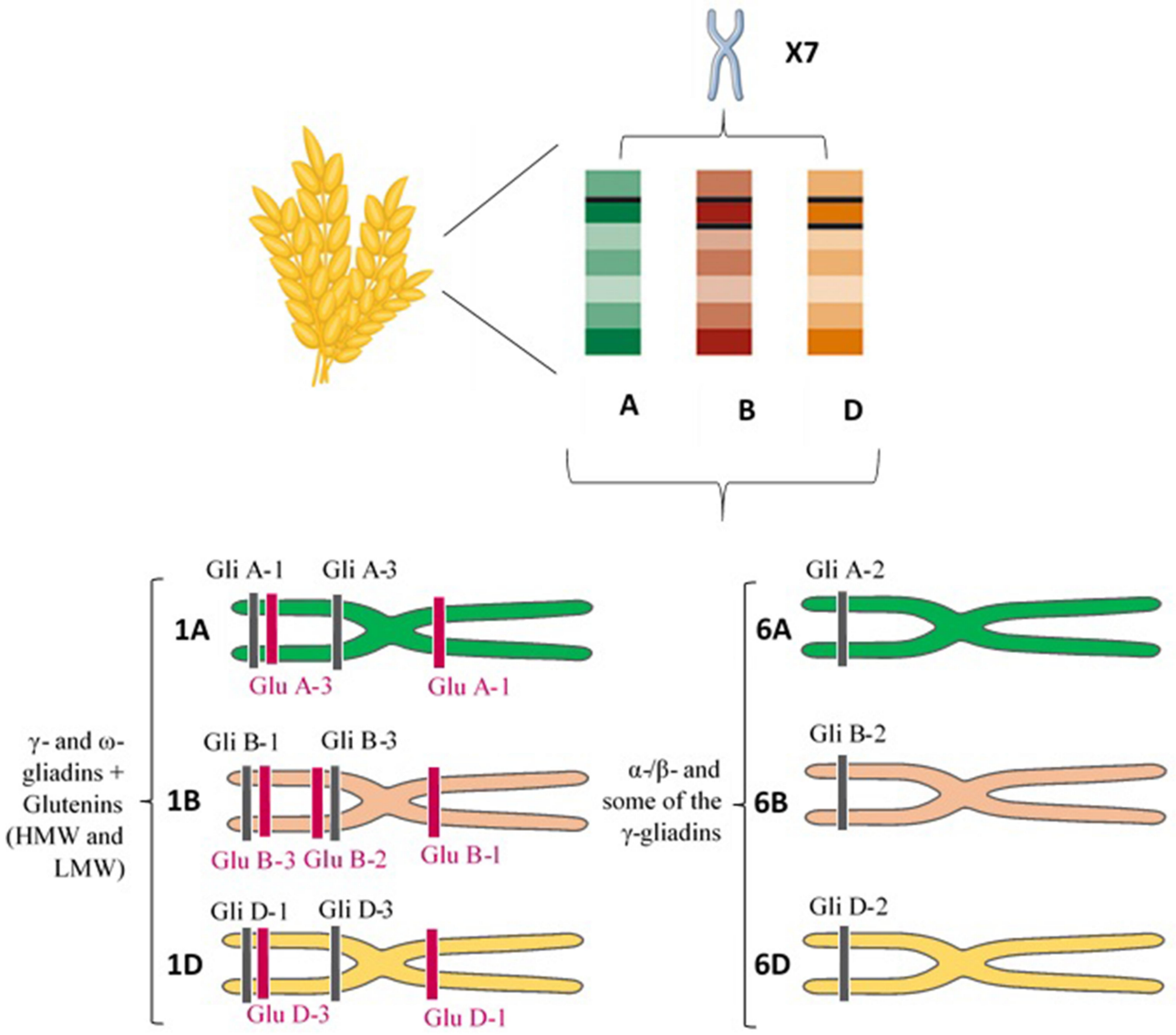

Figure 1 Chromosome site of different gluten constituents. Short arm of the homoeologous group 1 (Gli-A1, -B1 and -D1 loci) and 6 (Gli-A2, -B2 and -D2 loci) chromosomes encode γ - and ω-gliadins, and α-/β- and some of the γ-gliadins, respectively. Glu-A1, B1, and D1 loci (long arm) and Glu-A3, Glu-B3, and Glu-D3 loci (short arm) of the homoeologous group 1 chromosomes also encode the HMW-GS and LMW-GS subunits of glutenin, respectively.
Abbreviations: HMW, high molecular weight; LMW, low molecular weight.

some minor gliadin loci located on 1AS (*Gli-A3, -A5* and -*A6*), 1BS (*Gli-B3* and -*B5*) and 1DS (*Gli-D4* and -*D6*).[41]

α-gliadins are the most abundant storage proteins in cereal and several gene copy numbers (from 25 to 150) have been reported for them in haploid genome.[4,42–45] The α-gliadin genes originated from the D sub-genome of wheat and contribute the most immunogenic T-cell stimulatory peptides in wheat gluten for the 90% of CD patients who are positive for the HLA-DQ2.5 genotype.[42,46,47] However, the base substitution of glutamine codon (CAA) to a stop codon (TAA) can potentially cause inactivation of almost 50% of the α-gliadin genes.[20]

Glutenin Components and Genetic Characteristics

The glutenin, which represents 50% of total flour proteins, consists of huge polymeric proteins linked through inter- and intramolecular disulfide bonds and are among the largest protein molecules in nature.[2,14,36,48,49] Its separation by Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) shows high and low molecular weight (HMW-GS vs LMW-GS) subunits, which are 75 to 120 kDa and 30 to 74 kDa, respectively.[4,36,50] LMW-GS accounts for ~60% of the glutenins and has a greater and more favorable impact on the properties of the dough than

HMW-GS.[51,52] HMW-GS is grouped into x- and y-type subunits based on its electrophoretic mobility and molecular mass.[4,53] LMW-GS, which is similar to γ- gliadins in size and structure, is subdivided into B-, C-, and D-type subunits (differ from A, B and D genomes of wheat) basis on their isoelectric point (PI) and electrophoretic mobility (B-type is the major group).[36,52] The LMW-GS can also be classified based on the first N-terminal amino acid residue into m-type (Methionine), s-type (Serine) and i-type (Isoleucine) subclasses.[4,52]

The HMW-GS and LMW-GS genes are located on the *Glu-A1, B1*, and *D1* loci (long arm) and *Glu-A3, Glu-B3*, and *Glu-D3* loci (short arm) of the homoeologous group 1 chromosomes, respectively (Figure 1).[52,54] The *Glu-1* loci have multiple alleles and each of these loci includes two genes related to x- and y-type subunits.[54] The genes encoding LMW-GS are more complex and each *Glu-3* loci contain several genes and each gene has two or more alleles.[55,56] Genomic studies revealed that *Glu-3* loci are linked to *Gli-1* loci, and *Gli-1* loci encode LMW-GS in addition to γ - and ω-gliadin genes.[4] Accordingly, the C and D subunits of LMW-GS are very similar in sequence to α-/γ- and ω-gliadins, respectively.[36] LMW-GS and HMW-GS contribute peptides that are immunogenic in CD patients who carry the less common HLA-DQ2.2 and DQ8 genotypes.[57]

Gluten Protein and the Pathogenesis of Various GRDs

Celiac Disease (CD)

Studies reported that CD has a prevalence of approximately 1–3% in the general population worldwide especially in Western societies.[58,59] CD is a gluten-induced immune-mediated inflammatory disorder of the small intestine caused by an intolerance to dietary gluten. CD is limited to genetically predisposed individuals who carry HLA-DQ2.5, HLA-DQ8, HLA-DQ2.2 and/or rarely HLA-DQ7 haplotypes located on the short arm of chromosome 6.[60–64] The results of several Genome-wide association studies (GWAS), for example, most recently in a prospective study of 6010 children that carried HLA genotypes associated with increased risk of type-1 diabetes and CD, have also reported the role of non-HLA genes in CD presentation.[65] Other immunologic and environmental factors are also involved in the development of CD.[66] For instance, Caminero et al[57] demonstrated that opportunistic bacterial pathogens (such as P. aeruginosa) in duodenal biopsies from active CD patients could increase mucosal injury caused by immunogenic gluten-derived peptides in a mouse model through protease production and protease-activated receptor-2 (PAR-2) signaling.[57]

In general, the most immunogenic wheat gluten peptides in CD are derived from α-gliadins (Figure 2).[47,67] Some repetitive sequences include two or more overlapping immunodominant epitopes that bind to HLA-DQ2.5, while others include single copies of epitopes that bind HLA-DQ8 or HLA-DQ2.2 and stimulate effector memory CD4+ T cells.[68] According to standardized nomenclature, the most immunogenic fragment of α-gliadin for patients positive for HLA-DQ2.5 encompasses multiple copies of the overlapping DQ2.5-glia-α1a, DQ2.5-glia-α1b, and DQ2.5-glia-α2 epitopes.[68,69] Wheat α-gliadin also includes the subdominant DQ2.5-glia-α3 epitope, DQ8-glia-α1, DQ2.2-glia-α1 and the DQ2.2-glia-α2 epitopes, which are relevant in patients who are positive for HLA-DQ8, and/or HLA-DQ2.2.[40,68,70,71] Ozuna et al[46] studied six distinct types of α-gliadins in diploid and polyploid wheats through next-generation sequencing and Sanger sequencing. They found that α-gliadin sequences differed significantly in their frequencies and in the existence and abundance of CD immunogenic peptides. Their findings may help reduce the risk of CD incidence by the breeding/selection of wheat with low stimulatory properties.[46] Wheat ω-gliadin, however, includes two overlapping immunodominant epitopes, DQ2.5-glia-ω1 and DQ2.5-glia-ω2, that resemble DQ2.5-glia-α1a and DQ2.5-glia-α2, but stimulate a distinct population of CD4+ T cells and appear to be responsible for many cross-reactive CD4+ T cells activated by wheat, barley and rye.[47,72]

Gluten proteins are highly resistant to human digestive proteases (due to their high content of proline) and do not fully degrade during gastric and pancreatic digestion.[34,69] Two peptides that have attracted most attention and remain intact in the digestive process are the 33-mer (p55–87) and the 25-mer (p31–55) located in the α-gliadins encoded by the *Gli-D2* locus on chromosome 6D.[73,74] Of these two peptides, the 33-mer is the most digestion-resistant peptide with high immunogenic properties (contains DQ2.5-glia-α1a, DQ2.5-glia-α1b, and DQ2.5-glia-α2 epitopes).[75,76] During or after absorption of partially digested gliadin peptides into the lamina propria, specific glutamine residues of the 33-mer peptide are susceptible to pH-dependent transamidation (covalent cross-linking to free amines, for example, lysine residues

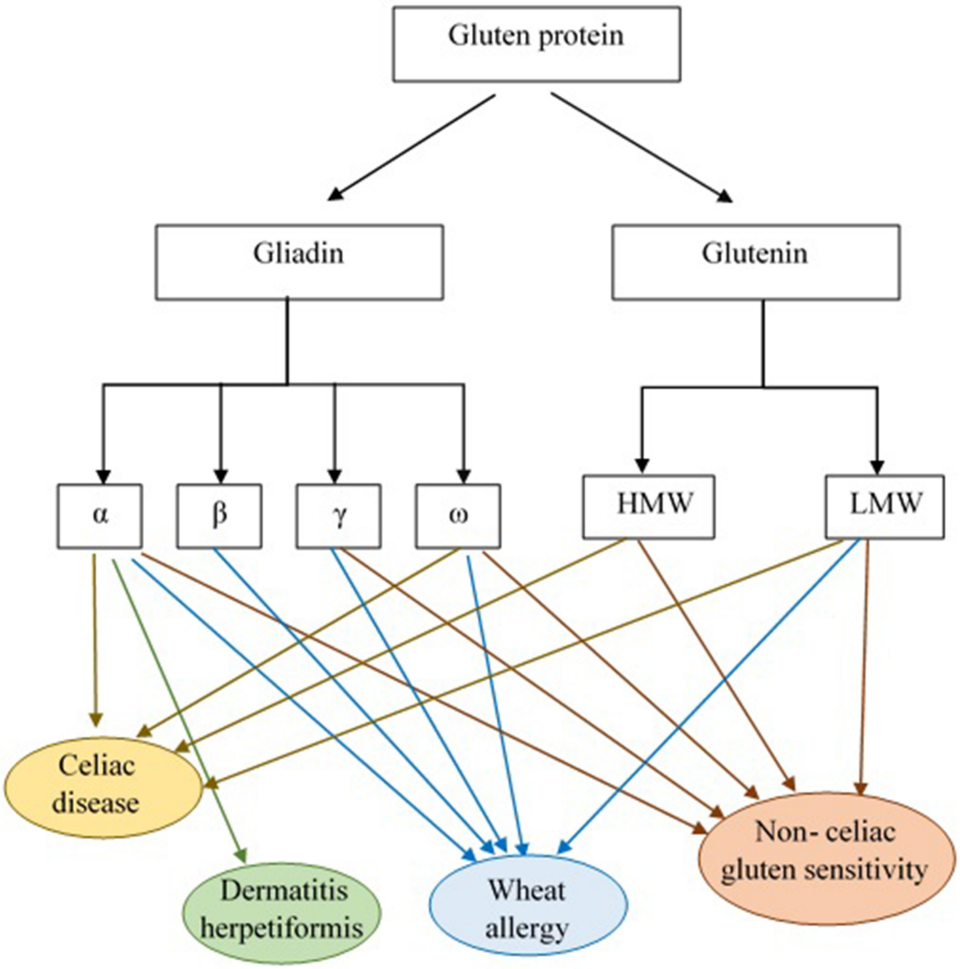

Figure 2 Gluten protein components and the role of its subgroups in GRDs pathogenesis. According to the results of studies α and γ-gliadins and glutenin are considered to be CD pathogenic responses eliciting factors. The α-gliadin fraction is also reported as DH immunological response triggering agent. Allergic reactivity to the α-/β-, γ- and ω-gliadin fractions and LMW-GS was observed in WA patients. Moreover, patients with NCGS revealed high levels of IgG antibodies against α-, γ- and ω-gliadin and glutenin.
Abbreviations: HMW, high molecular weight; LMW, low molecular weight.

in other proteins) or direct deamidation to glutamate through the action of extracellular tissue transglutaminase (tTG) expressed in inflamed host tissues.[77–79] Transamidation abolishes the immunotoxicity of gluten epitopes,[80] but direct deamidation enhances their affinity for HLA-DQ2.5 and is essential for their immunogenicity.[80–82] tTG-affected peptides are efficiently presented to CD4+ T-cells by the HLA-DQ molecules implicated in CD susceptibility. This results in gluten-specific CD4+ T cell activation with the secretion of pro-inflammatory cytokines like interleukin-2 (IL-2), IL-21 and interferon-gamma (IFNγ), antigen-non-specific activation of local cytotoxic CD8+ T cells,[83,84] and enterocyte injury and apoptosis, which ultimately contribute to the characteristic mucosal lesions and local inflammation associated with active CD. Gluten-stimulated CD4+ T cells also provide help for gliadin and tTG-specific B cells, and support antibody production by specific plasma cells.[84–87] Glutenin peptides are also implicated in T-cell responses.[87] In contrast, the in vitro effects of the α-gliadin 25-mer peptide include induction of IL-15 production from enterocytes and dendritic cells, and innate immune activation.[88] IL-15 promotes induction of inflammatory Th1 cell responses and also activation of cytotoxic CD8+ IELs leads to the development of the intestinal lesions.[89,90] The contribution of IL-15 to mucosal injury facilitated by induction of CD4+ T-cell immunity to gluten has been supported by a recently reported HLA-DQ8-expressing mouse model with overproduction of IL-15 in the gut epithelium and lamina propria, which develop gluten-dependent small intestinal villous atrophy mimicking human CD.[91]

Tye-din et al[47] in their follow-up study on HLA-DQ2.5 + CD patients screened for T cell–stimulatory gluten peptides in blood following a brief oral challenge with wheat, barley, and rye. They showed that α-gliadin 33-mer epitopes (DQ2-α-I and DQ2-α-II) are immunodominant only after the wheat challenge, while ω-gliadin/C-hordein–derived sequences encompassing DQ2-ω1/ω2 were the dominant T cell–stimulatory peptides in response to consumption of any of these cereals. Hence, they considered ω-gliadin/C-hordein–derived peptides as common T cell–stimulatory peptides in HLA-DQ2.5–associated CD patients.[47]

Nutrient malabsorption results from mucosal injury marked by villous blunting, crypt hyperplasia, increased intraepithelial lymphocytes (IELs) infiltrate, and immune-mediated enteropathy along with.[92] Gluten-induced systemic inflammation in CD is characterized by the presence of intestinal and/or extra-intestinal manifestations or it can even be completely asymptomatic.[59]

Dermatitis herpetiformis (DH) is one of the extra-intestinal presentations of CD that is accompanied by the development of papulovesicular pruritic skin rash on the extensor aspects of the limbs, sacral region, and buttocks.[59,93] As reported in previous studies, topical or intradermal use of gluten protein does not lead to DH formation and the incidence of this disorder is related to intestinal contact with gluten.[93] In fact, anti-tTG antibodies, made in response to gluten consumption, interact with the epidermal transglutaminase (ETG) enzyme and cause DH symptoms.[59] Allardyce and Shearman[94] in their study reported that cellular immune reactivity to the α-gliadin fraction was also observed in DH patients. Moreover, Huff et al[95] reported high levels of alpha-gliadin-specific antibodies in patients with DH (Figure 2). Therefore, more studies are required to evaluate the exact pathogenic fractions of gluten in DH.

Wheat Allergy (WA)

Wheat is one of the most common allergens and wheat allergy (WA) results from immunological adverse reactions to wheat ingredients, including water-soluble (albumin and globulin) and insoluble (glutenin and gliadin) proteins.[56,96–99] In fact, skin contact, inhalation or ingestion of wheat can lead to the occurrence of these allergic reactions.[97] Wheat-dependent exercise-induced anaphylaxis (WDEIA), where symptoms result from the ingestion of wheat in combination with physical exercise, and baker's asthma, that caused by inhalation of wheat flour, are classified as two commonly WA.[97,100] With a higher rate of reports in pediatrics, the prevalence of this disorder is reported to be between 0.5% and 1% in the world.[59,98]

Scientific reports have considered wheat ω-5 gliadin (fast ω-gliadin, Tri a 19), encoded by the *Gli-1* locus on chromosome 1B, as the major allergen part of gluten protein for various types of WA especially WDEIA (also known as ω5-gliadin allergy) (Figure 2).[101,102] Morita et al[103] reported that fast ω-gliadin is the main allergen for Japanese WDEIA patients. Moreover, results of the study conducted by Palosuo et al[104] showed that γ-70 and γ-35 secalins in rye and γ-3 hordein in barley that have structural homology and cross-reactivity with ω-5 gliadin could bind to IgE antibodies and elicit symptoms in WDEIA patients. Since WDEIA diagnosis is significantly delayed, Kennard et al[105] recommended the use of ω-5 gliadin-specific IgE testing for patients with unexplained anaphylaxis.[104] Moreover, according to Sandiford et al,[106] α- and fast ω-gliadin are also associated with baker's asthma allergic reactions (Figure 2).[105] Positive IgE responses to several other wheat grain proteins, such as α/β- (Tri a 21), γ-gliadins, low molecular weight (LMW) glutenin, and α-amylase /trypsin inhibitors (ATIs) are also reported (Figure 2).[98] For instance, Baar et al,[107] using a molecular discovery approach, found that Tri a 36, which belongs to the LMW-GSs (GluB3-23), is a wheat food allergen with IgE-reactive sequences.[56,107]

Following contact with allergens, secretion of IL-25 or IL-33 from epithelial cells leads to T helper type 2 (Th2) cell response activation and subsequently IgE antibodies production by B-cells.[108] This secreted IgE antibody bound to FceRI receptor on mast cells and basophils as well as to specific epitopes in wheat allergens resulting in the release of inflammatory chemical mediators such as histamine and platelet activator factor (PAF) (Figure 3).[25] As a result, allergic responses (such as itching, eczema, rhinitis, nausea) can be life-threatening and cause anaphylactic shock in some cases.[25]

Non-Celiac Gluten Sensitivity (NCGS)

Non-celiac gluten sensitivity (NCGS) or gluten sensitivity, which remains ill-defined, is a condition resulting from reactions to gluten-containing grains without IgE-mediated or T-cell-mediated responses.[59,109–111] Recent studies found that different non-gluten components of wheat flour such as ATIs, Fermentable Oligosaccharides, Disaccharides, Monosaccharides, and Polyols

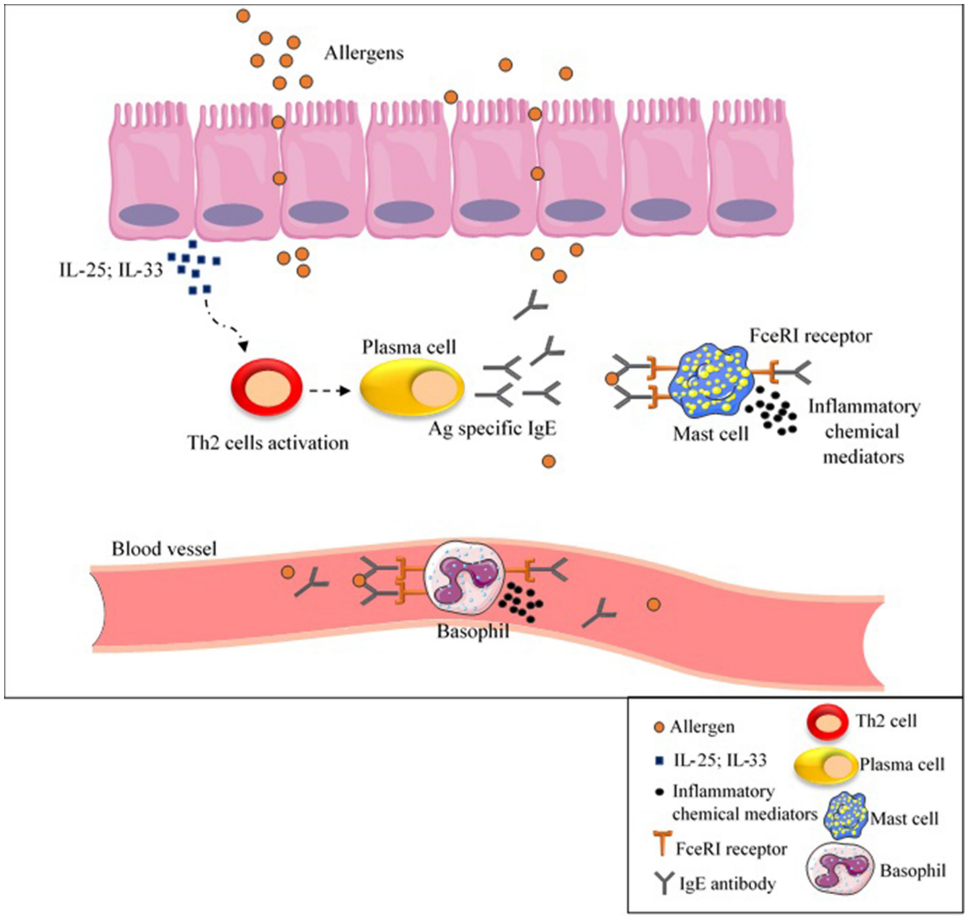

Figure 3 IgE- mediated wheat allergy. As a result of contact with allergens, IL-25 or IL-33 are secreted from epithelial cells, cause Th2 cell response activation and subsequently IgE antibodies production by B-cells. Inflammatory chemical mediators are released as a result of IgE antibody binding to FceRI receptor on mast cells and basophils as well as to specific epitopes in wheat allergens, causing allergic reactions.
Abbreviations: Ag, antigen; IgE, immunoglobulin E; IL, interleukin; Th2, T helper type 2.

(FODMAPs) causing irritable bowel syndrome might also contribute to NCGS.[59,112]

The precise pathogenesis of NCGS is still obscure; nevertheless, it has been reported that activation of the innate immune system could have a role in some patients with this condition.[113] In this regard, a decrease in T helper cell numbers and a reduction in regulatory T cell clones expansion and their cytokines production have been reported in mucosal biopsy specimens of NCGS patients.[114,115] Some early studies reported that HLA-DQ2 and/or -DQ8 genotypes could be over-expressed in NCGS, although these molecules are found in only around 50% of NCGS patients, which is not dissimilar to the general population.[56] NCGS is a widespread disorder with an estimated prevalence of 0.5% to 13%, which is more frequent in adult females.[116]

Given the lack of a definitive test to diagnose NCGS, which in some cases may be confused with CD or WA, several research studies focus on finding NCGS-specific serum biomarkers.[117,118] Tye-Din et al[119] in their study on CD and self-reported gluten sensitive (SR-GS) patients found that gluten challenge significantly increased IL-2, IL-8 and IL-10 serum levels in CD but not SR-GS patients (both groups had completely eliminated gluten from their diet before participating in the challenge). They concluded that cytokine assessment after acute gluten challenge could be used for distinguishing CD from SR-GS.[119] Uhde et al-[120] also reported a significant increase in anti-gliadin IgG1 and IgG3, and IgG2 and IgG4 subclasses in CD and NCGS patients, respectively. There was also a correlation between the IgG4 and IgG3 antibodies and serum concentration of Fatty acid-binding protein 2 (FABP2), which is an intestinal cell damage marker. They proposed that these components might be additional biomarkers to differentiate CD and NCGS.[120] The only presented case report by Vojdani and Perlmutter of a patient with NCGS and

autoimmunity revealed high levels of IgG antibodies against α-gliadin 33 and 17 mer, γ- and ω-gliadin and glutenin (Figure 2).[121]

The chronic symptoms ascribed to NCGS are similar to those of untreated CD with a wide range of intestinal and extra-intestinal presentation as highlighted in Salerno expert criteria.[4,117] In contrast, the double-blind sham-controlled gluten challenge that is low in FODMAPs does not generally induce measurable symptoms in patients self-reporting NCGS, whereas patients with treated CD typically experience acute upper gastrointestinal symptoms and show elevations in serum IL-2 within 2 hours.[122,123]

Wheat Genome Editing

Currently removing gluten from the diet is the only remedy to improve the symptoms of people prone to GRDs.[124] However, the addition of gluten to numerous food products and the high cost of gluten-free foodstuffs have made it difficult to strictly adherence to this diet.[15,27,125] As a result, several genetic studies have focused on breeding wheat with low immunological properties and preserved baking quality through biotechnological approaches. Numerous scientists believe that gene editing would be a definitive solution for GRDs; however, due to the variety of causal agents, it is not so easy to solve them all in this way[126–128] Biotechnological approaches are used for precise and organized modifying of specific genomic sequences through their different functions such as gene replacement, targeted gene knock-out and knock-in, etc.[129,130] Vasil et al[131] were the first group to successfully produce transgenic wheat plants in 1992 through the Bar gene transferring by biolistic particle bombardment method. The Bar gene encodes phosphinothricin acetyltransferase (PAT) enzyme, which is the cause of herbicide tolerance of plants.[131] RNA interference (RNAi) and CRISPR/Cas9 are the two recent biotechnology methods used in this regard.[126]

RNA interference (RNAi) is a post-transcriptional process present in almost all eukaryotic organisms and regulates the expression of protein-coding genes in a sequence-specific manner, which is capable of engineering novel phenotypes.[132,133] In fact, RNAi suppresses protein synthesis by using short double-stranded RNA (dsRNA) complementary to target mRNA and degrading that (silencing of the gene).[134] It has been shown that this method is very efficient in regulating gene expression in numerous plant systems.[135] Gil-Humanes et al[136] used this method to produce breads with up to 97% lower gliadin content (near gliadin-free). The results of their study showed that these reduced-gliadin breads had lower immunotoxicity compared to wild types, while physically no difference was observed between them. Additionally, the removal of gliadin leads to an increase in the number of lysine amino acids (due to the increase in glutenin content which contains more lysine residues) that increases the nutritional value of these breads.[136] Altenbach et al[137] suppressed the expression of ω-5 gliadins (as an important food allergen) in the US wheat cv Butte 86 using RNA interference technique. The results of their study showed that removing ω-5 gliadins from wheat did not affect flour functionality and had no effect on the expression of other grain proteins. Conversely, the removal of this part improved the dough properties and increased protein stability, indicates the negative role of ω-5 gliadins in flour quality.[137] In comparison, Altenbach et al[138] in their recent study used the same method to silence a subset of alpha-gliadin genes (containing CD epitopes) of wheat flour from the US spring wheat cultivar Butte 86. Analysing reactivities of IgG and IgA antibodies from patients with CD showed a significantly reduced immunoreactivity of the flour. However, their results showed a decrease in functional properties and dough strength in the transgenic lines. They proposed that the simultaneous removal of alpha and omega gliadins from wheat could be a more efficient approach in this regard.[138] In a comprehensive study, Barro et al[139] reported the effectiveness of seven RNAi containing plasmids with the ability to target α-, γ-, ω-gliadins, and LMW glutenin subunits in breeding non-toxic wheat variants without any CD epitopes.[139] Targeted gene knockdown by RNAi is a fast, low-cost and easy-to-perform method, however, while effective, it provides only transitory inhibition of gene function and may also have unpredictable effects on target genes leads to limited use of this method.[5,140]

There are some editing genome tools based on the effect of site-specific DNA-binding domain and the use of engineered nucleases, that can identify and edit a particular DNA sequence, including zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs).[5] The clustered regularly interspersed short palindromic repeats (CRISPR)/Cas system, especially CRISPR-associated protein 9 (CRISPR/Cas9), is a widely used prokaryotic nuclease-based target gene precise editing tool, which known as an effective alternative to ZFNs and TALENs.[5,141,142] CRISPR/Cas9,

identified as the most popular genetic engineering technique, causes genome modifications by delivering to plant cells and expressing there.[129,142,143] In this method, the single guide RNA (sgRNA) directs the caspase to the target DNA sites and helps breeding low immunogenic epitopes containing plants with greater specificity.[144] The first successful use of CRISPR-Cas9 system was to knock out TaMLO gene (Mildew-resistance locus O) in wheat protoplasts which leads to improved disease resistance that brings the importance of the CRISPR/Cas9 system to promote important traits.[145] Sánchez-León et -al,[146] using two single guide RNAs (sgAlpha-1 and sgAlpha-2) targeted coding sequence for α-gliadin genes, show that CRISPR/Cas9 technology could be used for providing wheat lines with reduced immunoreactivity.[145] Jouanin et al[147] in their pilot study reported the efficacy of CRISPR/Cas9, using six sgRNA sequences, in mutating α- and γ-gliadin gene copies and preventing them from triggering the human immune system.[147]

The Court of Justice of the European Union (CJEU) in July 2018 considered any crop with altered genetic material caused by new plant breeding techniques (unnatural changes) as genetically modified organisms (GMOs), which are subjects within the scope of EU law and can be used. This judgment, although supported by some, has also provoked criticism, which led to the formation of the new European Commission that may result in the EU's GMO legislation change.[148]

Conclusion

As lifelong adherence to a gluten-free diet remains a challenge for GRDs patients, it seems that increasing attention to the immunogenetic properties of gluten constituents is an essential element in improving the condition of patients. In this regard, technologies have been designed that can reduce the immunogenicity properties of gluten by promising a genomic editing approach. Although these techniques mostly work precisely on the target gene, it is important to note that changes in the expression of one gene how affect the expression of other genes? It can be said that one of the reasons for not including these manipulated products in patients' diets is the lack of a clear answer to this question. Therefore, it is suggested that future studies in this regard consider all genome-editing results (either genetic or metabolic changes) to ensure the created product compatibility with food safety conditions.

Abbreviations

ATIs, Alpha-amylase/Trypsin Inhibitors; CD, Celiac Disease; CRISPR, Clustered Regularly Interspersed Short Palindromic Repeats; DH, Dermatitis herpetiformis; ETG, Epidermal Transglutaminase; FABP2, Fatty Acid-Binding Protein 2; FODMAPs, Fermentable Oligosaccharides, Disaccharides, Monosaccharides, and Polyols; g, Gram; GWASs, Genome-Wide Association Studies; HMW, High Molecular Weight; IELs, Intraepithelial Lymphocytes; IgE, Immunoglobulin E; IL, Interleukin; kDa, Kilodalton; LMW, Low Molecular Weight; NCGS, Non-Celiac Gluten Sensitivity; P, Proline; PAF, Platelet Activator Factor; PAT, Phosphinothricin Acetyltransferase; PI, Isoelectric Point; Q, Qlutamine; RNAi, RNA interference; sgRNA, single guide RNA; SR-GS, Self-Reported Gluten Sensitive; TALENs, Transcription Activator-Like Effectors Nucleases; Th2, T helper type 2; tTG, Tissue Transglutaminase; WA, Wheat Allergy; WDEIA, Wheat-Dependent Exercise-Induced Anaphylaxis; ZFNs, Zinc-Finger Nucleases.

Author Contributions

All authors made a significant contribution to the work reported in all of the following areas: took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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