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# Wheat grain allergies: an update on wheat allergens

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## KEY WORDS

*Wheat allergens, water/salt-soluble fraction, gluten allergens,  $\omega$ 5-gliadins, wheat isolates*

## SUMMARY

*Wheat grain is a major staple of our diet. However, proteins derived from wheat grain have been implicated in both respiratory and food allergies, as well as in contact hypersensitivity. Numerous wheat allergens are present in the different fractions of wheat grain:  $\alpha$ -amylase/trypsin inhibitor and lipid transfer protein are found in the water/salt soluble fraction, and  $\omega$ 5-gliadins and LMW-glutenins have been detected in the gluten fraction. This review discusses what is currently known about wheat grain proteins and allergens. The type of IgE-binding profiles (allergens or even epitopes) in patients with wheat food allergy as a function of age, symptoms, or genetic variability of wheat cultivars provides interesting and useful data for developing hypoallergenic foods as well as new tools for diagnostic and therapeutic methods.*

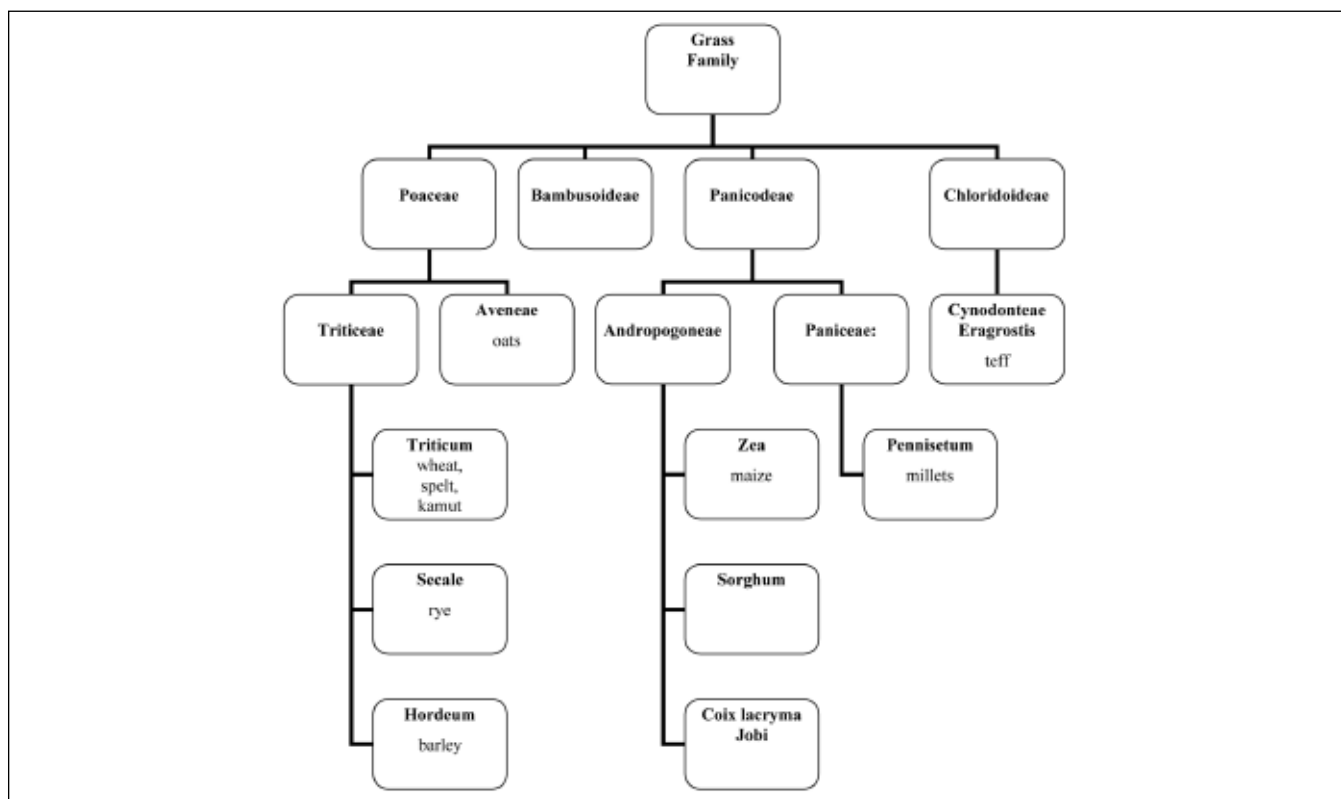
## Introduction

Wheat is the most widely cultivated cereal and represents an important food and dietary protein source worldwide. Wheat proteins can be either ingested or inhaled in the form of raw flour, which can lead to adverse reactions. These adverse reactions cover a broad spectrum of disorders due to different affected pathways and show a variety of clinical manifestations: wheat gluten enteropathy (celiac disease), T-cell-mediated intestinal inflammation, dermatitis herpetiformis, a blistering skin eruption, respiratory allergy to wheat pollen or flour, and wheat grain food allergy. Food allergies include various clinical features, including atopic dermatitis in children, and exercise-induced anaphylaxis, anaphylactic shock, and/or chronic urticaria in adults. This review focuses on what is currently known about the characteristics and structures of wheat grain proteins and wheat grain allergens.

## Wheat grain

Cereals are members of the grass family (*Poaceae*) that produce edible dry fruits, known as seeds or kernels. Cereal production is the highest in the world with total annual grain yields estimated in 2006 to be 2020 million tons (mt) (1). Although this includes a large variety of cereal species, three specific cereals together account for >70% of total production: maize (694 mt estimated yield in 2006), rice (631 mt estimated yield in 2006) and wheat (592 mt estimated yield in 2006). Other cereals include barley, sorghum, millets (which consists of a number of small-seeded tropical species), oats and rye, in order of decreasing total production, and finally rare species such as teff (Fig. 1) (2).

The most highly consumed cereals by man include rice and wheat (*Triticum spp.*). Among all the cereal grains, wheat is unique because wheat flour alone has the ability

**Figure 1** - Schematic representation of cereal species and grass family classification

to form a dough that exhibits the rheological properties required for the production of leavened bread and for the wider diversity of foods that have been developed to take advantage of these attributes. Wheat grain is a staple food used to make flour for leavened, flat and steamed breads, cookies, cakes, pasta, noodles and couscous, as well as for fermentation to make beer, alcohol, vodka or biofuel. There are many cultivated species of wheat (Tab. 1), of

which two have currently a real economic impact: (A) the common wheat (*Triticum aestivum*) is more typically cultivated in the high latitudes (for example in France, in Canada, in Ukraine). It is largely used to make flour for the bread market; (B) the durum wheat (*Triticum durum*) is cultivated especially in hot and dry zones (southern Europe, for example southern France or Italy). The durum wheat is very rich in gluten, and is used to produce semolina flour and pasta products.

**Table 3** - Major cultivated species of wheat

Common Names	Species	Genomes
<b>Bread Wheat</b>	<i>Triticum aestivum</i>	Hexaploid
<b>Durum wheat</b>	<i>Triticum durum</i>	Tetraploid
Einkorn	<i>Triticum monococcum</i>	Diploid
Emmer	<i>Triticum dicoccum</i>	Tetraploid
Spelt	<i>Triticum spelta</i>	Hexaploid
Kamut® or QK-77	<i>Triticum polonicum</i> or <i>T. durum</i>	Tetraploid

Wheat species in bold are species most widely cultivated in the world

### Wheat grain proteins

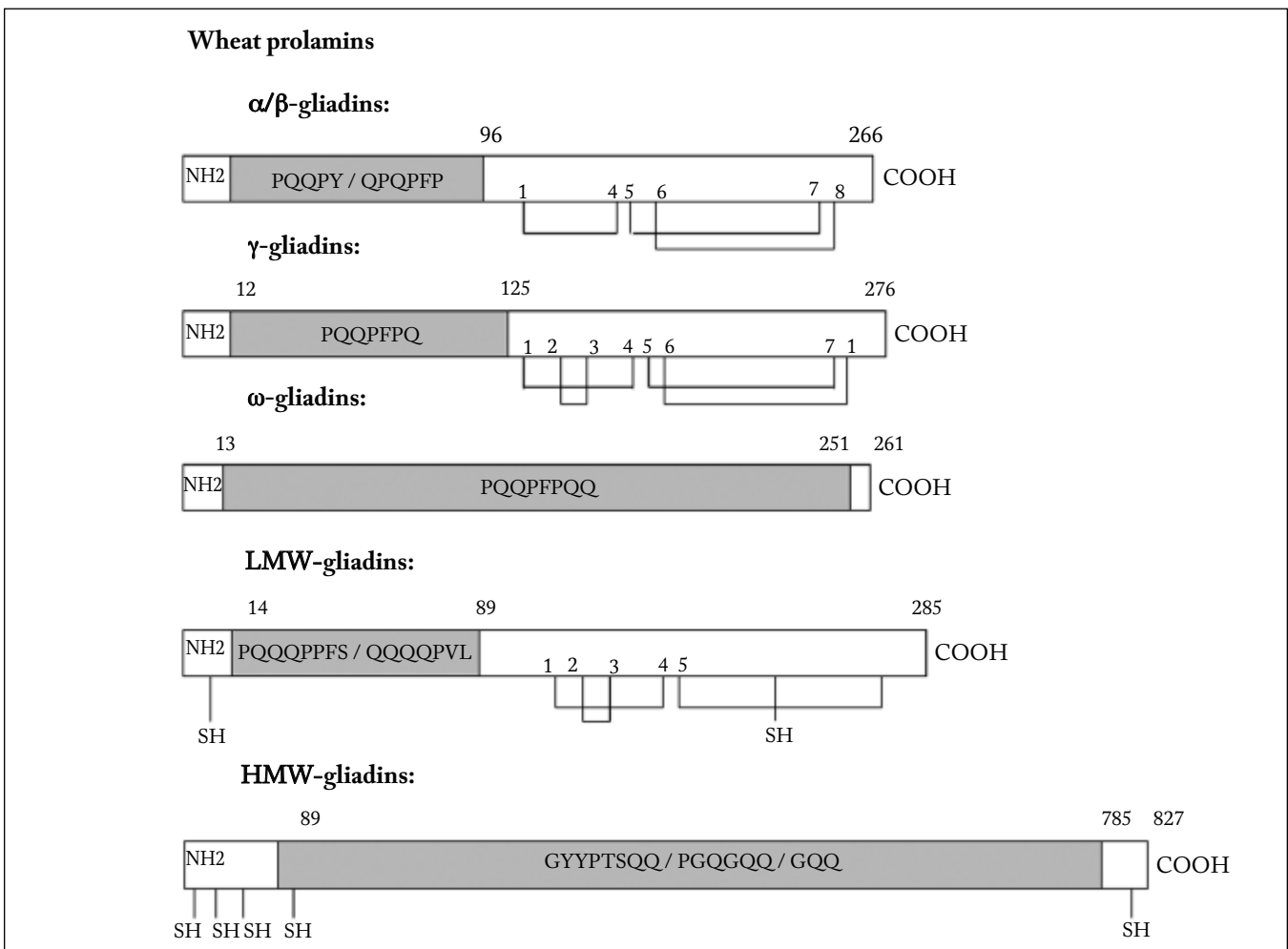
Wheat grains do not contain many proteins (about 10-15% of dry weight) as compared to legume seeds (about 25-30%). Osborne developed a classification of plant proteins based on their solubility in various solvents (3): water (albumins), dilute salt (globulins), aqueous alcohol (gliadins), and dilute alkali or acid (glutenins) solutions. The water/salt-soluble albumins and globulins are mainly structural proteins and metabolically active enzymes, which contribute about 50% of the total lysine content in

the seed proteins (4). Some proteins have been identified as belonging to the family of  $\alpha$ -amylase/trypsin inhibitors and others as part of the lipid transfer family. Several proteins have unknown functions and are not yet well characterized (5, 6). High molecular weight albumins and certain globulins (triticins) are considered to have an additional storage function.

Proteins, between 13 and 18 kDa, corresponding to  $\alpha$ -amylase inhibitors and proteins at 37 and 62 kDa corresponding to a peroxidase precursor and a serine carboxypeptidase, respectively, were identified by N-terminal sequencing of the albumin fraction (7). Some authors have also identified several proteins in the globulin fraction (20, 37, 38 and 54 kDa) homologous to barley globulins (2, 8).

The water/salt-insoluble gliadins and glutenins, together known as prolamins or gluten proteins, are the major storage proteins of the wheat grain and are well characterized (9, 10). The name prolamins reflects the fact that these proteins are unusually rich in proline and glutamine (accounting for 30-70% of total amino acids which is typically observed in cereal species) that are found in highly repetitive sequence motifs (2). The main features of prolamins are the high occurrence rate of genetic polymorphisms (one wheat variety can contain more than 50 different prolamins), their similar organization into repetitive and non-repetitive domains and the presence of extensive sequence homologies between the different gliadins and glutenins (Fig. 2). Moreover, wheat pro-

**Figure 2** - Schematic structures of some wheat prolamins (according to Shewry et al. 1994 and 2002, Anderson et al. 2001 and Hsia et al. 2001) (2, 102-105). Repetitive domains are shaded and conserved cysteine residues (1 to 8, from  $\alpha/\beta$ -,  $\gamma$ -gliadins and LMW glutenins) forming an intramolecular disulphide bridge are connected by a black line and the corresponding numbers 1-8. SH denotes the positions of cysteine residues in LMW and HMW glutenins not implicated in disulphide bridge



lamins share a great degree of sequence and structural homology with the corresponding proteins in rye and barley (2, 9, 11).

The ethanol-soluble gliadin group comprises over 40 structurally similar monomeric proteins, which account for 30-40% of wheat grain proteins. They are classified as  $\alpha/\beta$ -,  $\gamma$ - and  $\omega$ -types on the basis of their electrophoretic mobility in acid-polyacrylamide gel electrophoresis (PAGE) (12). The molecular weights (MW) of  $\alpha/\beta$ - and  $\gamma$ -gliadins range from 30-40 kDa and those of  $\omega$ -gliadins from 40-50 kDa, although their apparent MW in sodium dodecyl sulfate (SDS)-PAGE is much higher (55-75 kDa) (10, 13). Slow- and fast-moving  $\omega$ -gliadin components are observed in acid-PAGE and were designated by Kasarda et al. (14), as  $\omega_1$ ,  $\omega_2$  (slow-moving) and  $\omega_5$  (fast-moving) as a function of their mobility and their N-terminal sequences.

The alkali or acid-soluble glutenins account for 35-40% of total proteins and correspond to polymeric proteins linked by intermolecular disulfide bridges (10). They are composed of high molecular weight (HMW) subunits (apparent MW in SDS-PAGE ranges from 80-120 kDa) and of low molecular weight (LMW) subunits. LMW glutenins are further divided into three groups of subunits: the B-group (subunits of 42-51 kDa), the C-group (30-40 kDa), including subunits related to  $\alpha/\beta$ - and  $\gamma$ -gliadins, and the D-group (60-75 kDa) containing  $\omega$ -gliadin-like subunits.

The availability of complete amino acid sequences of various prolamins has allowed the redefinition of their classification in relation to structural and evolutionary relationships (11). This system of classification assigns all of the prolamins of the Triticeae (wheat, barley and rye) family to three broad groups: sulfur-rich, sulfur-poor and HMW prolamins (Tab. 2) (2). The sulfur-rich prolamins (LMW glutenins,  $\alpha$ -gliadins and  $\gamma$ -gliadins) are composed of an N-terminal domain that contains proline and glutamine-

rich repeats and a C-terminal non-repetitive domain with even numbers of cysteine residues that form intra-chain disulfide bonds. The non-repetitive domains have been suggested to be rich in  $\alpha$ -helices (15). In contrast,  $\omega$ -gliadins consists almost entirely of repeats and are characterized by a low content of sulfur-containing amino acid residues and a lack of cysteine residues.

### Wheat grain allergens

Wheat grain proteins are involved in the three routes of sensitization: inhalation, contact and ingestion (16). Depending on the route of allergen exposure and the underlying immunologic mechanisms, wheat grain allergy can appear as occupational asthma and rhinitis, contact urticaria or classic food allergy affecting the skin, gut, and/or respiratory tract, or as exercise-induced anaphylaxis (17-22).

Baker's asthma is one of the most common forms of occupational asthma. Baker's asthma is an occupational disease affecting 4-10% of bakery workers in European countries (23). Food allergy, defined as adverse immune response to food proteins, affects as many as 6% of young children and 3% to 4% of adults (24). Any protein-containing food may induce an allergic reaction. Wheat is among the six foods, identified by Codex Alimentarius, responsible for approximately 90% of food allergies in children, and in recent years has been increasingly recognized as a cause of food-dependent exercise-induced anaphylaxis (FDEIA) (21). Wheat allergens have been involved in 6% of life-threatening food anaphylaxis between 2002 and 2004 (25) in France, in which exercise could have been an aggravating factor. However, no fatal cases of exercise-induced wheat-related anaphylaxis has been reported to date, despite the frequency of allergy to wheat (25). In Iran, wheat is reported as the most fre-

**Table 2** - Classification of wheat grain prolamins (characteristics and groups) adapted from Shewry et al. 2002 (2)

Prolamins (70% of total wheat grain proteins)		
Sulfur-rich (70-80% of prolamins)	Sulfur-poor (10-20% of prolamins)	HMW prolamins (6-10% of prolamins)
$\alpha/\beta$ -gliadins $\gamma$ -gliadins B- and C-groups of LMW-GS 65-90 kDa	$\omega$ -gliadins D-group of LMW-GS 30-45 kDa	HMW glutenin subunits 30-75 kDa

LMW-GS: LMW glutenin subunits

quent causes of anaphylaxis for children (26). Wheat allergy appears more frequently in Northern (27) than in Southern Europe. In France, wheat ranks as the 8<sup>th</sup> most frequent food allergen in children and as the 12<sup>th</sup> in adults (28). It represents 20% of the food allergy clinical pediatric population in the study of Sicherer (29) and 14% in the study of Niggemann (30). In the study of Moneret-Vautrin et al, it represents 10.9% of children and 25% of adults (20). In the cohort of the Isle of Wight on six-year-old children, wheat was the third key allergen giving positive food challenges (31). In contrast, in an American study on food allergy in children, wheat-related food allergy was present in only 2.5% of the cases (32, 33). These numbers could be underestimated because they represent only the most severe cases where hospital care was necessary.

Clinical manifestations of wheat allergy are similar to those of other food allergies, with symptoms at the level of the skin, the gastrointestinal tract and the respiratory tract (17). Clinical patterns in children and adults are different. The main symptoms in children are atopic dermatitis (AD), either alone or associated with respiratory symptoms and digestive problems (27, 34-38). In adults, various clinical features have been identified including FDEIA, anaphylactic shock, angioedema, irritable bowel syndrome, eosinophilic oesophagitis, rare cases of ulcerative colitis or buccal aphthosis (22, 39-48). Wheat may be introduced very early in the diet, around the fifth month after birth. Moreover, sensitization might occur much earlier through maternal milk, in which the presence of non-degraded gliadins have been reported in breast-feeding mothers not following a specific diet (49, 50). On the contrary, Poole et al. (51), observed that delaying initial exposure to cereal grains until after 6 months may increase the risk of developing wheat allergy. Wheat-dependent exercise-induced anaphylaxis (WDEIA) was mostly observed in adults, and sometimes in children (48, 52, 53). The diagnosis, however, is difficult, as well as the exercise level necessary to induce the symptoms, and the wheat quantities are very variable (53-55).

#### *Water/salt-soluble allergens*

Several water/salt soluble proteins have been described as allergens:  $\alpha$ -amylase inhibitors, serpin (serine proteinase inhibitor), acyl-coenzyme A oxidase, fructose-biphosphate aldolase and wheat flour peroxidase (56-58). Among them, the  $\alpha$ -amylase/trypsin inhibitor family, which include several 12-17 kDa proteins, are consid-

ered to play a significant role as allergens for individuals with baker's asthma (56, 59, 60). This protein family is also a relevant sensitizing allergen for wheat food allergic patients (45, 61, 62). A large panel of allergens comprised between 12 and 70 kDa belonging to water/salt soluble proteins have been also described in wheat allergies (16, 63-67). We also have shown that water/salt soluble proteins appeared as significant allergens in 60 children and adult patients with wheat food allergy confirmed by double-blind, placebo-controlled food challenge (DBPCFC) (68). Wheat lipid transfer proteins (LTPs) have been identified as allergens for wheat allergic patients (62, 68, 69). LTP1 (9kDa) and LTP2 (7kDa) were recognized by specific IgE from patients with wheat food allergy; this IgE population also demonstrated *in vitro* cross reactivity to wheat, barley and maize LTPs (69). Recently, a novel cross reactive cereal allergen family, the wheat thioredoxins, has been identified as being related to baker's asthma (70).

#### *Prolamin or gluten (gliadins and glutenins) allergens*

All gliadin and glutenin protein fractions have been described as wheat grain allergens in several studies (16, 19, 62, 67, 71-73). Alpha- and  $\omega_5$ -gliadins are also associated with baker's asthma (74). In a previous study, we observed IgE binding to  $\alpha/\beta$ -,  $\gamma$ - and  $\omega$ -gliadins in addition to LMW glutenins for wheat allergic children and adults (66). In a more extensive study with 60 patients, we showed that different allergenic profiles could be detected in wheat food allergy, as a function of patient age and symptoms manifested (68). Indeed,  $\alpha/\beta$ - and  $\gamma$ -gliadins (with some proteins of water/salt-soluble fraction) appeared to be more important allergens for children with AD with or without asthma, while  $\omega_5$ -gliadins were major allergens for adults with WDEIA and/or anaphylaxis (100%) or urticaria (55%). Only 23% of patients with AD and 8% of those with AD and asthma reacted to  $\omega_5$ -gliadins. B-type LMW glutenin subunits also was identified as significant allergens in adult anaphylaxis cases (62, 68) but also in children (75). In all cases, HMW glutenins were only minor allergens. The specific role of  $\omega_5$ -gliadins in WDEIA has been demonstrated by several studies (48, 55, 76). Palosuo et al. revealed that  $\omega_5$ -gliadins are also allergens in children with an immediate-type allergy to wheat or with wheat-induced anaphylaxis (77, 78). The same group showed that  $\omega_5$ -gliadins induced release of histamine from the basophils of patients with WDEIA but not from those of controls (48). The authors also pre-

**Table 3** – Summary of major IgE-binding epitopes identified on prolamins allergens

Proteins	Sequences	Symptoms	References
Gliadins	QQPFP/PQQPF	AD	(84)
$\gamma$ -gliadins	QQLVPQ/QQSFPQ	WDEIA	(87)
$\omega_2$ -gliadins	QQPIPQQ/QQFPQQ	WDEIA	(87)
$\omega_5$ -gliadins	QQXPQQ/QQSPEQQ	WDEIA, A, U	(85, 87)
LMW glutenins	QQQPP	AD	(82, 83)
HMW glutenins	QQPGQ/QQPGQGQQ/QQSGQGQ	WDEIA	(86)

A: Anaphylaxis; AD: Atopic Dermatitis; U: Urticaria; WDEIA: Wheat dependent-exercise induced anaphylaxis.

X: being F, I, L, Y or S.

sented data hypothesizing that tissue transglutaminase (tTG) in the intestinal mucosa of patients could be activated during exercise. Indeed, tTG-mediated cross-linking of a pepsin-trypsin digested  $\omega_5$ -gliadin causes a marked increase in binding to IgE (79). Moreover, serum gliadin levels are correlated with clinical symptoms induced by exercise and aspirin in patients with WDEIA (80). The authors suggested that exercise and aspirin facilitate allergen absorption from the gastrointestinal tract. Recently, recombinant forms of  $\omega_5$ -gliadins demonstrated similar IgE-binding abilities (81).

Several studies have identified IgE-binding epitopes on prolamins allergens and for wheat allergic patients with different symptoms (Tab. 3). A QQQPP motif has been identified as an IgE-binding epitope in LMW glutenin for patients with AD (82, 83). The same group reported IgE-binding abilities of QPFP and PQQPF motif in gliadin sequence for patients with AD (84). Another study restricted to WDEIA cases described several linear epitopes among  $\omega_5$ -gliadin repeats (QQXPQQ with X being I, F, S, Y or L and QQSPEQQ) (85). In this study, LMW glutenins were also detected by IgE in some patients with WDEIA. Several sequences are known for these proteins and some of them contain the motifs QQLPQQQ or QQFPQQQ in their repetitive domains that could cross-react with  $\omega_5$ -gliadin epitopes. Three IgE-binding epitopes within the primary sequence of HMW glutenin have also been observed for Japanese patients with WDEIA (86). We also reported several IgE-binding linear epitopes on  $\alpha/\beta$ -,  $\gamma$ - and  $\omega$ -gliadins ( $\omega_2$ - and  $\omega_5$ -gliadins) for wheat allergic patients (87). Patients (mainly adults) with anaphylaxis, urticaria or WDEIA, showed strong IgE-binding to sequential epitopes of the repetitive domains of gliadins. Among these repetitive domains, 2 immunodominant epitopes on  $\omega_5$ -gliadin with a consensus motif of the type QQX<sub>1</sub>PX<sub>2</sub>QQ (X<sub>1</sub> being L, F,

S or I and X<sub>2</sub> Q, E or G) were identified. It is thus interesting to see that IgE of wheat allergy patients of Japanese or French descent recognized the same epitopes. The absence of IgE linear epitopes in children with atopic eczema/dermatitis syndrome leads to the hypothesis that specific responses occur to conformational epitopes.

#### *Gluten modified allergens*

The principal properties of the wheat grain reside primarily in the gluten-forming storage proteins of its endosperm. Wheat gluten proteins form a continuous viscoelastic network when flour is mixed with water to form dough (88). While rice is frequently consumed by humans without any processing except cooking, wheat is almost always consumed after processing (89). Bread, pastries, pasta, and noodles are just the few examples of processed products. However, wheat gluten actually exhibits low solubility in aqueous solution (2). This causes limited applications of wheat gluten on various types of food, as solubility is the main characteristic of proteins selected for use in liquid foods and beverages. Furthermore, solubility is closely related to other functional properties of proteins such as foaming, emulsification, and gelling ability (88). This explains why many processes are used to improve solubility of wheat gluten, such as hydrolysis and/or deamidation (89).

These biochemical modifications on gluten proteins are currently being used by various industries (food, cosmetics, pharmaceutical, ...). In Japan and several Asian countries, these derivatives or modified wheat products are traditionally used as seasoning. These wheat products could induce a reduction of wheat allergenicity by decreasing the molecular mass of native wheat allergens after enzymatic or acid hydrolysis or deamidation (89-92). On the other hand, these treatments on native proteins could also

uncover allergenic motifs or create neoallergens. Wheat hydrolyzed proteins have been described as being responsible for immediate hypersensitivity reactions such as contact urticaria or anaphylaxis (93-95). Wheat isolate, corresponding to a concentrate of gluten proteins which have been submitted to heat and acid treatments causing a partial deamidation, have been implicated in some cases of allergy restricted to wheat isolates, in the absence of reactivity to unmodified wheat itself (16, 96, 97).

## Conclusion

The route of sensitization appears to be important in the development of wheat allergy. Patients with baker's asthma are mostly sensitized to raw flour particles inhaled via the respiratory mucosa, whereas patients with food allergy are primarily sensitized to heat-treated and digested wheat proteins absorbed through the gastrointestinal epithelium. Indeed, Lauriere et al. (98), also suggest that state and route of exposure to very similar structures probably orientate the pattern of epitope reactivity and clinical manifestations. Nevertheless, review of the literature indicates that a large variety of wheat grain proteins have been identified as allergens in all pathologies studied. The type of IgE-binding profile in patients with wheat food allergy is correlated to age and symptoms that manifest. The elucidation of the major IgE-binding epitopes on wheat allergens could provide a useful tool for developing hypoallergenic foods as well as new diagnostic techniques and immunotherapy for patients with wheat grain allergies. Monitoring of the development of wheat allergies in patients is critical in determining relationships between wheat protein epitopes and allergy persistence. Indeed, this approach has already proven useful for egg and milk allergies (99, 100). Furthermore, studies on genetic variability of wheat cultivars represent a new and interesting approach for developing ways of decreasing allergenicity of wheat products (101).

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