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Evaluation of Allergenicity on a ω-5 Gliadin-Deficient Cultivar in Wheat-Dependent Exercise-Induced Anaphylaxis

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ABSTRACT

Purpose: ω-5 gliadin is the major allergen that causes wheat-dependent exercise-induced anaphylaxis (WDEIA). Recently, a missing mutant wheat cultivar at 1B chromosome *Glu-B3* and closely linked *Gli-B1 loci* was bred. This cultivar (ω5D) has a deficiency in ω-5 and γ-gliadins as well as some low-molecular-weight glutenins. We evaluated specific immunoglobulin E (sIgE) reactivity of the ω5D in WDEIA patients compared to wild-type cultivar.

Methods: Serum samples from 14 WDEIA and 7 classic wheat allergy patients were used to compare the allergenicity of ω5D and wild-type cultivars using immunoglobulin E immunoblotting, enzyme-linked immunosorbent assay (ELISA), and ImmunoCAP inhibition assays.

Results: Immunoblotting revealed that ω5D extracts had less sIgE binding to gliadins and glutenins in WDEIA sera than wild-type extracts. Immunoblot inhibition assay for gliadin sIgE reactivity also showed that ω5D gliadins had less allergenicity than wild-type gliadins. ELISA inhibition assay showed stronger allergenicity of gliadins than glutenins, although they had cross-reactivity. This assay also showed that the 50% inhibitory concentrations (IC50) of ω5D extracts against gliadin- or glutenin-sIgE reactivity were approximately 4-fold higher in WDEIA patients than those of wild-type extracts. The inhibition capacity of ω5D gliadins against recombinant ω-5 gliadin-sIgE reactivity was also lower in WDEIA patients than that of wild-type.

Conclusions: The allergenicity of the ω5D cultivar is markedly lower for WDEIA patients in the sIgE inhibition tests. These results suggest that the ω5D cultivar may be a safe alternative for WDEIA patients.

Keywords: Food allergy; wheat; food-dependent exercise-induced anaphylaxis; gliadin; glutenin

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INTRODUCTION

Wheat can induce various allergic diseases, such as classic food allergy, occupational asthma/ allergic rhinitis, and wheat-dependent exercise-induced anaphylaxis (WDEIA), through respiratory, gastrointestinal, or skin exposure.[1] Wheat proteins can be classified into 4 major Osborne fractions: water-soluble albumins, salt solution-soluble globulins, alcohol-soluble gliadins, and glutenins which are alcohol-soluble in the presence of urea and reducing agents (e.g., dithiothreitol).[2] The water- and salt solution-soluble proteins are involved in metabolism and have been recognized as the major allergens associated with the pathogenesis of classic food allergy and occupational wheat flour asthma/allergic rhinitis.[3] Glutenins and gliadins, especially ω-5 gliadin, are the proteins that are the major allergens involved in WDEIA.[4-7]

The composition of Osborne fractions varies considerably depending on the species, variety, and growing conditions of wheat cultivars. A quantitative study demonstrated the variability in the compositions of 13 wheat cultivars: water- and salt solution-soluble proteins (10%–34%), gliadins (48%–62%), and glutenins (15%–48%).[2,8] Gliadins are classified into different types based on their properties. The α/β- and γ-types are more common and comprise 28%–33% and 23%–31% of gliadins, respectively. However, the ω-type of gliadins are less common, and the ω-1/2 and ω-5 types constitute 4%–7% and 3%–6% of total gliadins, respectively.[8,9] Previous studies have shown that ω-5 gliadin as well as low-molecular-weight (LMW) and high-molecular-weight (HMW) glutenin subunits are the major allergens of WDEIA.[5,7,10] The Korean National Institute of Crop Science bred a wheat cultivar with a defect in the chromosome 1B *Glu-B3* locus, which encodes LMW glutenins, and closely linked *Gli-B1* locus, which encodes ω-5 and γ-gliadins. So, this new cultivar (ω5D) has selective deletions in the ω-5 gliadins as well as some i- and m-type LMW glutenins and γ-gliadins, which are important in the pathogenesis of WDEIA. However, it still expresses the α/β-, ω-1/2, some γ-gliadins, and a small amount of ω-5 gliadins from the 1D chromosome, as well as HMW and some LMW glutenin subunits.[9,11]

In this study, we evaluated the allergenicity of a wheat cultivar deficient in the ω-5 gliadin and especially m-type LMW glutenin in WDEIA patients using *in vitro* immunoglobulin E (IgE) immunoblot and inhibition IgE immunoassays with enzyme-linked immunosorbent assay (ELISA) and ImmunoCAP.

MATERIALS AND METHODS

Enrolled patients

This study included the serologic evaluation of 14 WDEIA and 7 classic wheat allergy (CWA) patients (**Table 1**). All WDEIA patients exhibited compatible clinical symptoms of anaphylaxis associated with exercise, and recombinant ω-5 gliadin-specific immunoglobulin E (sIgE) reactivity (Phadia f416). CWA patients had clinical features of immediate-type (within 1 hour from exposure) anaphylaxis or angioedema/urticaria, allergic rhinitis symptoms by occupational exposure, and they exhibited whole water/salt-soluble wheat extract sIgE (Phadia f4) reactivity but not recombinant ω5 gliadin-sIgE reactivity (**Table 1**). We considered sIgE-positive when the sIgE level was measured by ImmunoCAP (Thermo Fisher Scientific, Waltham, MA, USA) and was higher than 0.34 kU/L. For serologic evaluations, we used individual patient's serum samples or pooled samples from 5 WDEIA patients (patient numbers 1, 3, 4, 9, and 10 in **Table 1**) as well as 5 CWA patients (patient numbers 15, 16, 17, 18, and 19 in **Table 1**) throughout this study. This study was approved by the Institutional Review

Table 1. Clinical features of enrolled wheat allergy patients

Variables	No.	Age	Sex	Wheat, f4 sIgE (kUA/L)	ω-5 gliadin, f416 sIgE (kUA/L)	Clinical features	Coexisting allergic diseases	Diagnosis
WDEIA f4 (−), f416 (+)	1	47	M	0.06	5.47	BP↓, U, by running	-	WDEIA
	2	24	F	0.24	8.00	BP↓, Dyspnea, U, by exercise	Chronic U	WDEIA
	3	66	M	0.18	1.36	BP↓, LOC, U, by fast walking	-	WDEIA
	4	68	M	0.33	1.98	BP↓, U, by exercise	-	WDEIA
	5	41	F	0.19	5.73	A, BP↓, LOC, U, by exercise or NSAID	-	WDEIA
	6	44	F	0.14	3.19	LOC, U, by running	-	WDEIA
	7	61	M	0.19	1.05	A, BP↓, U, by exercise	AR	WDEIA
	8	55	M	0.20	16.70	Arrest, BP↓, Dyspnea, LOC, by OPT	-	WDEIA
WDEIA f4 (+), f416 (+)	9	45	M	4.02	32.10	Abdominal pain, U, by walking	-	WDEIA
	10	36	F	0.98	7.05	A, BP↓, Dyspnea, U, by fatigue	AR	WDEIA
	11	23	M	0.40	2.61	BP↓, Diarrhea, Dyspnea, U, by exercise	-	WDEIA
	12	27	F	2.11	27.40	BP↓, Dyspnea, U, by exercise	-	WDEIA
	13	23	F	0.92	10.70	BP↓, U, by walking	-	WDEIA
	14	49	M	1.59	14.10	LOC, U, by running	-	WDEIA
Classic wheat allergy f4 (+), f416 (−)	15	23	F	0.44	0.00	A, BP↓, U, without exercise	BW allergy	Anaphylaxis
	16	18	M	0.45	0.00	Dyspnea, U, by exercise	-	Anaphylaxis
	17	18	M	1.51	0.00	A, U, without exercise	BW/kiwi anaphylaxis	A, U
	18	44	M	8.38	0.00	Sneezing, by wheat flour	Asthma, chronic U	Occupation AR
	19	44	M	1.21	0.10	U, without exercise	Oak pollinosis, OAS	U
	20	23	M	1.05	0.07	U, without exercise	-	U
	21	32	F	0.41	0.00	Dyspnea, U, without exercise	-	Anaphylaxis

sIgE, specific immunoglobulin E; WDEIA, wheat-dependent exercise-induced anaphylaxis; BP, blood pressure; U, urticaria; LOC, loss of consciousness; A, angioedema; NSAID, non-steroidal anti-inflammatory drug; OPT, oral provocation test; AR, allergic rhinitis; BW, buckwheat; OAS, oral allergy syndrome.

Boards of Yonsei University Health System (Approval No. 4-2017-1258). All enrolled patients submitted their consent for study enrollment.

Wheat cultivars and preparation of wheat extracts

This ω5D wheat cultivar was selected from a double haploid population produced from 2 different Korean wheat cultivars: *Triticum aestivum* L. cv. Keumkang and *Triticum aestivum* L. cv. Olgeuru. Keumkang is a hard cultivar used for bread and noodle, and Olgeuru is soft wheat for noodles. This ω5D cultivar has missing *Glu-B3* and closely linked *Gli-B1* loci, which results in deficiency of ω-5, γ-gliadins, s-type-, i-type-, and m-type-LMW glutenins.[9,11,12]

We extracted gliadins and glutenins from the ω5D and wild-type bread flour (*T. aestivum* ssp. *aestivum* L; CJ CheilJedang, Seoul, Korea) purchased from the market. Wheat flour was incubated with 50% propanol for 20 min at 65°C and was then centrifuged at 10,000 ×g for 5 minutes. The supernatant, which consisted of the extracted gliadins, was removed and stored. The centrifuged pellet was incubated with 50% propanol, 0.08 M Tri-HCl (pH 8.0), and 2% dithiothreitol and was then centrifuged at 10,000 ×g for 5 minutes. The resulting supernatant consisted of the extracted glutenins and was stored at −70°C until further use. The water/salt solution-soluble extract was prepared in phosphate-buffered saline (PBS) at 4°C for 24 hours after defatting with ethyl ether. The extract was centrifuged at 13,000 ×g for 15 minutes at 4°C, and the supernatant was dialyzed with distilled water. All dialysates were syringe-filtered (0.22-μm pore size; Merck, Darmstadt, Germany) and lyophilized. The wheat extract protein concentrations were measured using the Bradford assay (Bio-Rad, Hercules, CA, USA).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and IgE immunoblot reactivity/inhibition assays

The extracts were separated by SDS-PAGE on 10% gels and then either stained with Coomassie blue dye or transferred to polyvinylidene difluoride membranes. After blocking with 3% skim

milk, membranes were incubated overnight at room temperature with pooled serum samples (1:4 dilution) from WDEIA and CWA patients. For IgE antibody detection, membranes were incubated with an alkaline phosphatase-conjugated goat anti-human IgE (1:1,000) (Sigma-Aldrich, Burlington, MA, USA) for 1 hour. The colorimetric reaction was developed with the nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl-phosphate system (Promega, Madison, WI, USA). For the immunoblot inhibition assay, pooled serum samples from the WDEIA and CWA patients were pre-incubated with 20 μg glutenins or gliadins isolated from the wild-type and ω5D cultivars at room temperature for 2 hours. Then, the IgE reactivity was detected, as described above.

ELISA and ImmunoCAP inhibition assays

Wild-type glutenins and gliadins were coated onto a microplate (Corning Inc., Corning, NY, USA). After blocking with 3% skim milk, the plate was incubated overnight with pooled serum samples (1:10 dilution) from WDEIA and CWA patients that were pre-incubated with various concentrations (0.016–50 μg/mL) of glutenins or gliadins from the wild-type and ω5D cultivars. IgE antibodies were detected by incubating with a biotinylated goat anti-human IgE (1:1,000) (Vector, Burlingame, CA, USA), followed by incubation with a streptavidin-peroxidase conjugated antibody (1:1,000) (Sigma-Aldrich). Color development was initiated by adding 3,3′5,5′-tetramethyl-benzidine substrate (Kirkegaard & Perry Laboratories, Gaithersburg, MD, USA). Absorbance was measured at 450 nm after stopping the enzyme reaction by adding 0.5 M H_2SO_4. The inhibition percentage was calculated by (1 − absorbance with inhibitors/absorbance without inhibitors) × 100. Additionally, the ImmunoCAP inhibition assay was performed for whole water/salt-soluble wheat allergen and recombinant ω-5 gliadin sIgE reactivity. Serum samples were pre-incubated with various concentrations of glutenins or gliadins isolated from the wild-type and ω5D cultivars. Subsequently, sIgE reactivity against the whole-wheat water/salt soluble allergen and recombinant ω-5 gliadin were measured by the ImmunoCAP system (Thermo-Fisher Scientific, Uppsala, Sweden). The inhibition percentage was calculated as described above.

RESULTS

SDS-PAGE and IgE immunoblotting analyses with WDEIA and CWA patient serum samples

SDS-PAGE analysis of glutenins and gliadins extracts showed apparent differences in protein composition compared to the water-soluble extract. There were also differences in the band patterns of the glutenins and gliadins found in the wild-type and ω5D cultivar extracts. IgE immunoblots after incubation with the pooled serum samples also showed marked differences between the WDEIA and CWA patients. The sIgE antibodies of the WDEIA patients were mainly bound to glutenins and gliadins, whereas those of the CWA patients were primarily bound to water/salt-soluble proteins in addition to glutenins and gliadins. Anti-glutenins and anti-gliadins IgE immunoblotting patterns differed between the wild-type and ω5D cultivars in the WDEIA patients (**Fig. 1**). The anti-glutenins and anti-gliadins IgE binding were attenuated in ω5D than in wild-type cultivar, especially at 35–50 kDa.

To compare the allergenicity of these wheat cultivars, we performed an IgE immunoblot inhibition assay using pooled serum samples (**Fig. 2**). The degree of inhibition in glutenins sIgE reactivity was similar between the glutenins from the wild-type and ω5D extracts (**Fig. 2A**). However, the ω5D extract exhibited less inhibition of gliadin sIgE reactivity compared to the gliadins from the wild-type cultivar extract in the WDEIA patients (**Fig. 2B**).
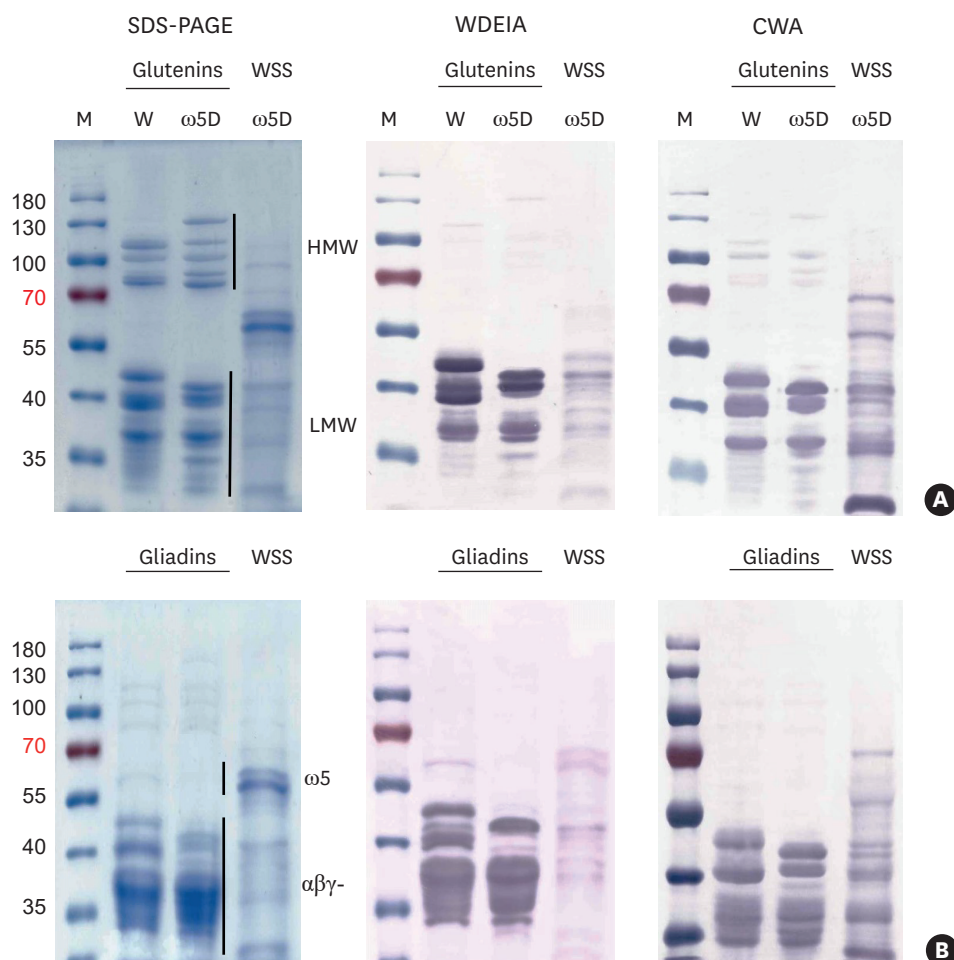
Fig. 1. SDS-PAGE and IgE immunoblots of glutenins (A), gliadins (B), and water- and salt soluble wheat extracts. Immunoblotting was performed after incubation with pooled serum samples from WDEIA (n = 5) and CWA patients (n = 5).

SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel; IgE, immunoglobulin E; WDEIA, wheat-dependent exercise-induced anaphylaxis; CWA, classic wheat allergy; HMW, high molecular weight glutenin subunits; LMW, low molecular weight glutenin subunits; M, marker; W, wild-type; WSS, water- and salt-soluble extract; αβγ-, α-, β-, γ-gliadins; ω5, ω-5 gliadins; ω5D, ω-5 gliadins and LMW glutenins deficient.

IgE immunoblot analysis with individual serum samples from 14 WDEIA and 7 CWA patients revealed that individual WDEIA samples exhibited less sIgE binding to the glutenins (**Fig. 3A and B**) and gliadins (**Fig. 3C and D**) isolated from the ω5D compared to those from the wild-type cultivar.

ELISA inhibition assay for evaluation of gliadins and glutenins cross-reactivity

The above data showed that the WDEIA patients also exhibited significant sIgE reactivity to glutenins. Therefore, we evaluated the presence of any cross-reactivity between gliadins and glutenins using an ELISA inhibition assay against wild-type gliadins- and glutenins-sIgE reactivity. As expected, the wild-type gliadins-sIgE inhibition experiment showed that the allergenicity of wild-type gliadins is stronger than that of ω5D in the aspect of the maximum inhibition % (93.9% vs. 81.1%) and 50% inhibitory concentration (IC50: 0.6 vs. 4.2 μg/mL). The wild-type glutenins also inhibited the wild-type gliadin-sIgE reactivity, with a 74.9% maximum inhibition and IC50 of 7.3 μg/mL (**Fig. 4A**).
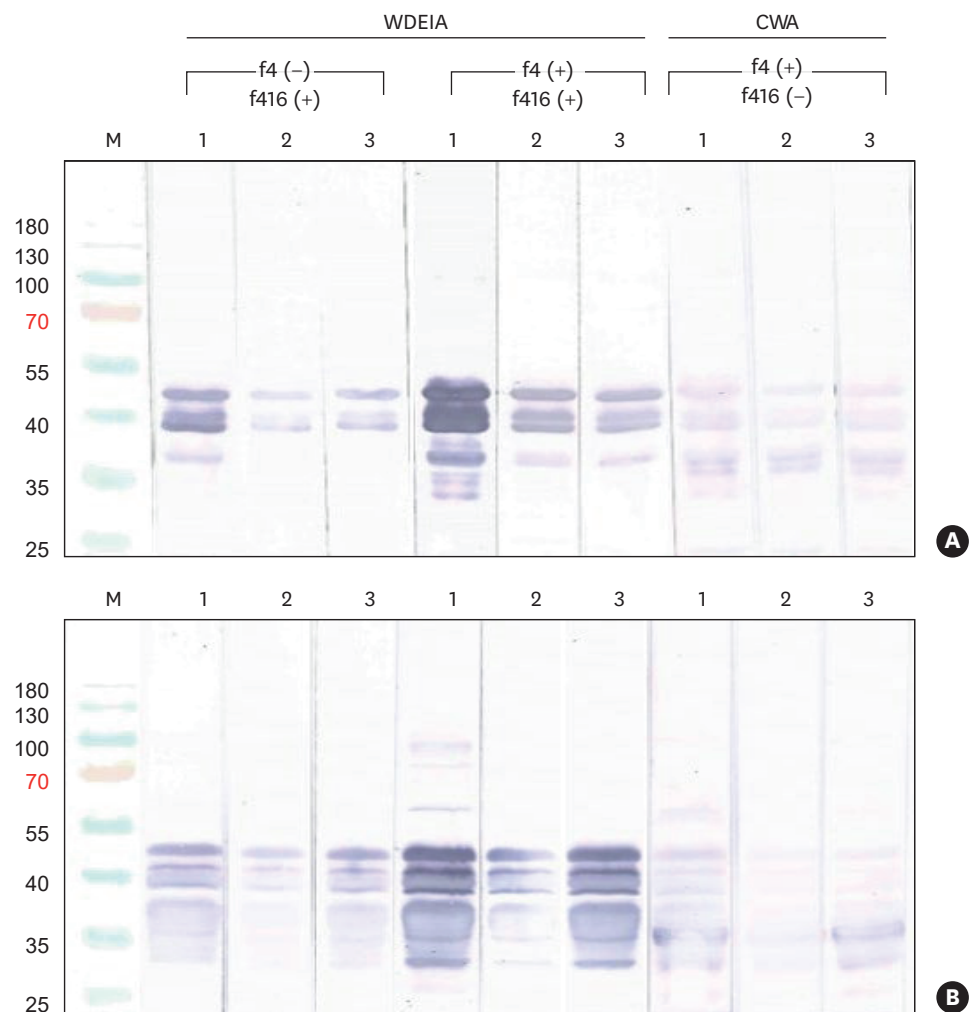
Fig. 2. IgE immunoblotting inhibition assay for wild-type glutenins (A) or gliadins (B) extracts after incubation with the pre-incubated pooled serum samples from WDEIA patients. Lane 1: positive control, Lane 2: immunoblot with 20 µg of wild-type glutenins or gliadins used as inhibitors, Lane 3: immunoblot with 20 µg of the glutenins or gliadins isolated from the ω5D cultivar used as inhibitors.

IgE, immunoglobulin E.

The wild-type glutenins-sIgE inhibition experiment also showed that the allergenicity of wild-type glutenins was stronger than that of ω5D in the aspect of maximum inhibition % (90.5% vs. 66.7%) and IC50 (5.6 vs. 13.8 µg/mL). Interestingly, the wild-type gliadins evoked a much stronger inhibition on wild-type glutenins-sIgE reactivity than the wild-type glutenins. Both the wild-type gliadins and glutenins extracts inhibited glutenins sIgE reactivity with a 98.3% and 90.5% maximum inhibition, but the IC50 values were 0.4 and 5.6 µg/mL, respectively, suggesting that the gliadins exhibited a 14-fold stronger allergenicity compared to that of the glutenins (**Fig. 4B**).

ELISA and ImmunoCAP inhibition assays for evaluation of the allergenicity of the ω5D and wild-type cultivars

To quantify the allergenicity, we performed ELISA and ImmunoCAP inhibition assays using the previously described pooled serum samples from the WDEIA and CWA patients. The maximum and IC50 values against wild-type glutenins- or gliadins-sIgE reactivity were determined by
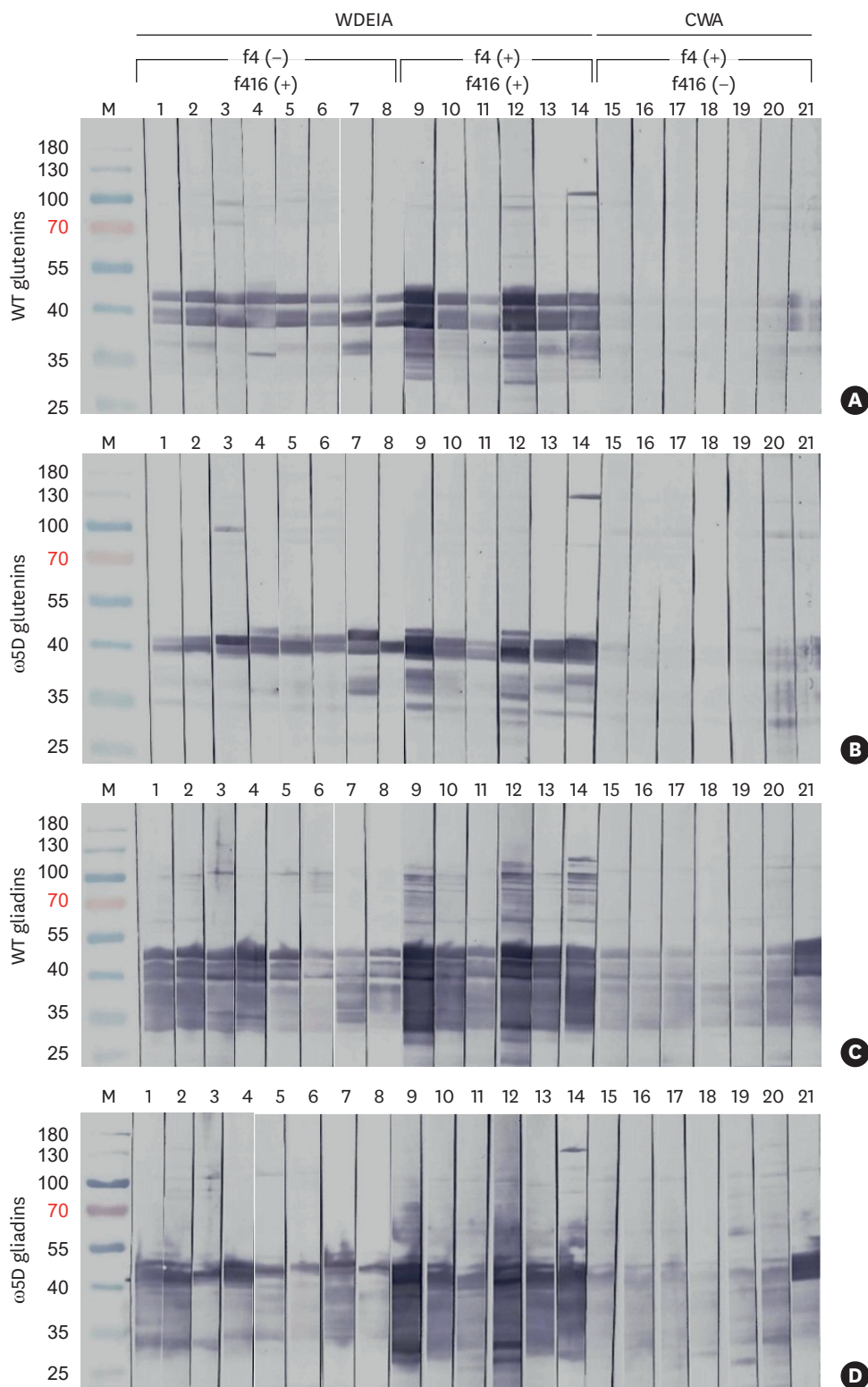
Fig. 3. IgE immunoblotting for glutenins (A, B) and gliadins (C, D) from wild-type or ω5D cultivars with the individual serum samples of WDEIA and CWA patients.

IgE, immunoglobulin E; WDEIA, wheat-dependent exercise-induced anaphylaxis; CWA, classic wheat allergy.
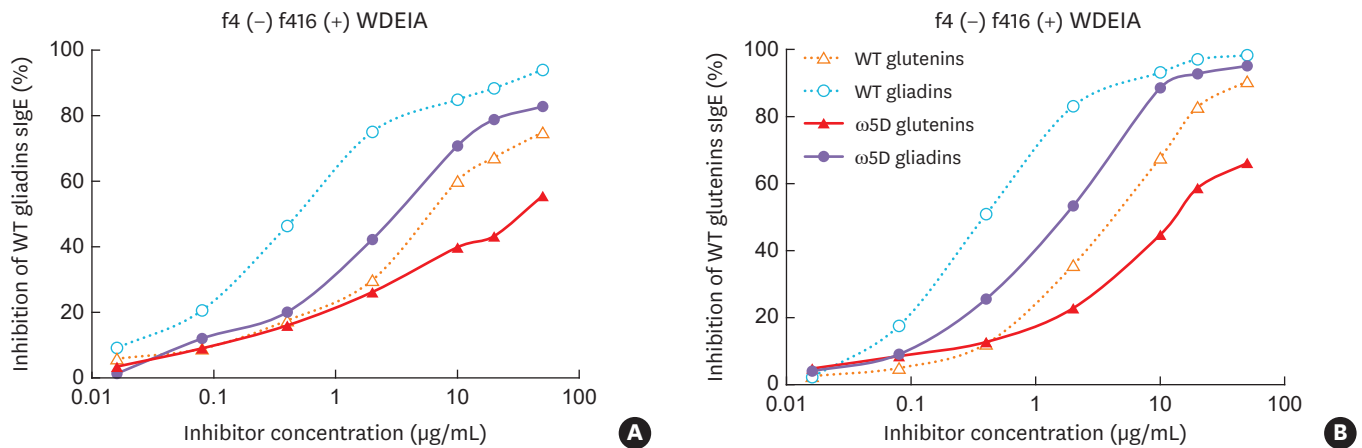
Fig. 4. ELISA inhibition assay for WT gliadins (A) and glutenins (B) sIgE reactivity after incubation with pre-incubated pooled serum samples from WDEIA patients. Gliadins and glutenins from WT and ω5D cultivars were used as inhibitors.
ELISA, enzyme-linked immunosorbent assay; WT, wild-type; sIgE, specific immunoglobulin E; WDEIA, wheat-dependent exercise-induced anaphylaxis.

the ELISA inhibition assay (**Table 2**). The IC50 of the ω5D cultivar for wild-type glutenins- and gliadins-sIgE reactivity was approximately 4-fold higher in the WDEIA patients than that of the wild-type cultivar (**Table 2, Fig. 5A, B, D, and E**). However, the IC50 values of the wild-type and ω5D cultivars were not different in the CWA patients (**Table 2, Fig. 5C and F**).
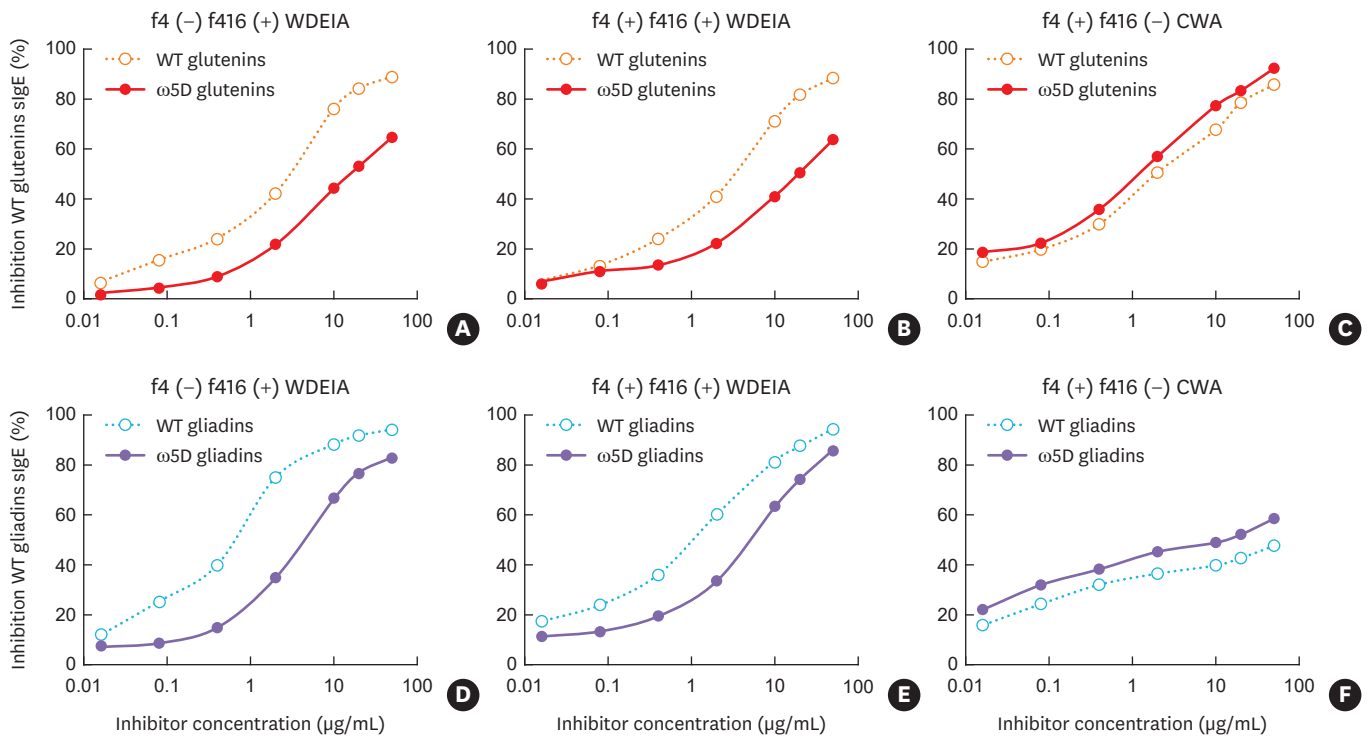

Fig. 5. ELISA inhibition assay for WT glutenins (A-C) and gliadins (D-F) sIgE reactivity with the corresponding inhibitors from the WT and ω5D cultivars. Pooled serum samples from WDEIA and CWA patients were used.
ELISA, enzyme-linked immunosorbent assay; WT, wild-type; sIgE, specific immunoglobulin E; WDEIA, wheat-dependent exercise-induced anaphylaxis; CWA, classic wheat allergy.

Table 2. Maximum inhibition percentages and IC50 values of wild-type glutenins, wild-type gliadins, and recombinant ω-5 gliadin (Phadia f416) sIgE reactivity in response to wild-type and ω5D cultivar extracts

Variables	Wild-type glutenins sIgE				Wild-type gliadins sIgE				Recombinant ω-5 gliadin sIgE			
	Max inhibition (%)		IC50 (µg/mL)		Max inhibition (%)		IC50 (µg/mL)		Max inhibition (%)		IC50 (µg/mL)	
	Wild-type	ω5D	Wild-type	ω5D	Wild-type	ω5D	Wild-type	ω5D	Wild-type	ω5D	Wild-type	ω5D
WDEIA f4 (−), f416 (+)	89.0	64.8	3.8	16.5	94.3	83.0	0.9	5.9	48.3	30.9	-	-
WDEIA f4 (+), f416 (+)	88.4	63.7	4.3	18.3	93.1	85.2	1.2	7.6	48.4	35.6	-	-
Classic wheat allergy f4 (+), f416 (−)	85.7	92.3	2.0	1.5	47.6	58.5	-	-	ND	ND	ND	ND

IC50, 50% inhibition concentration; sIgE, specific immunoglobulin E; ND, not done.

ImmunoCAP inhibition assay for whole water/salt-soluble wheat extract-sIgE reactivity was also performed against the glutenins and gliadins isolated from the wild-type and ω5D cultivars. There were no differences in the inhibition curves based on the types of wheat proteins used for inhibition in the WDEIA and CWA patients (**Fig. 6A-D**). Next, we performed the ImmunoCAP inhibition assay against recombinant ω-5 gliadin-sIgE reactivity, using a maximum concentration of 800 µg/mL of gliadins extracts as the inhibitor in the pooled serum sample of the WDEIA patients. We observed different patterns in inhibition curves based on the type of wheat proteins used as the inhibitors. The maximum percentage of
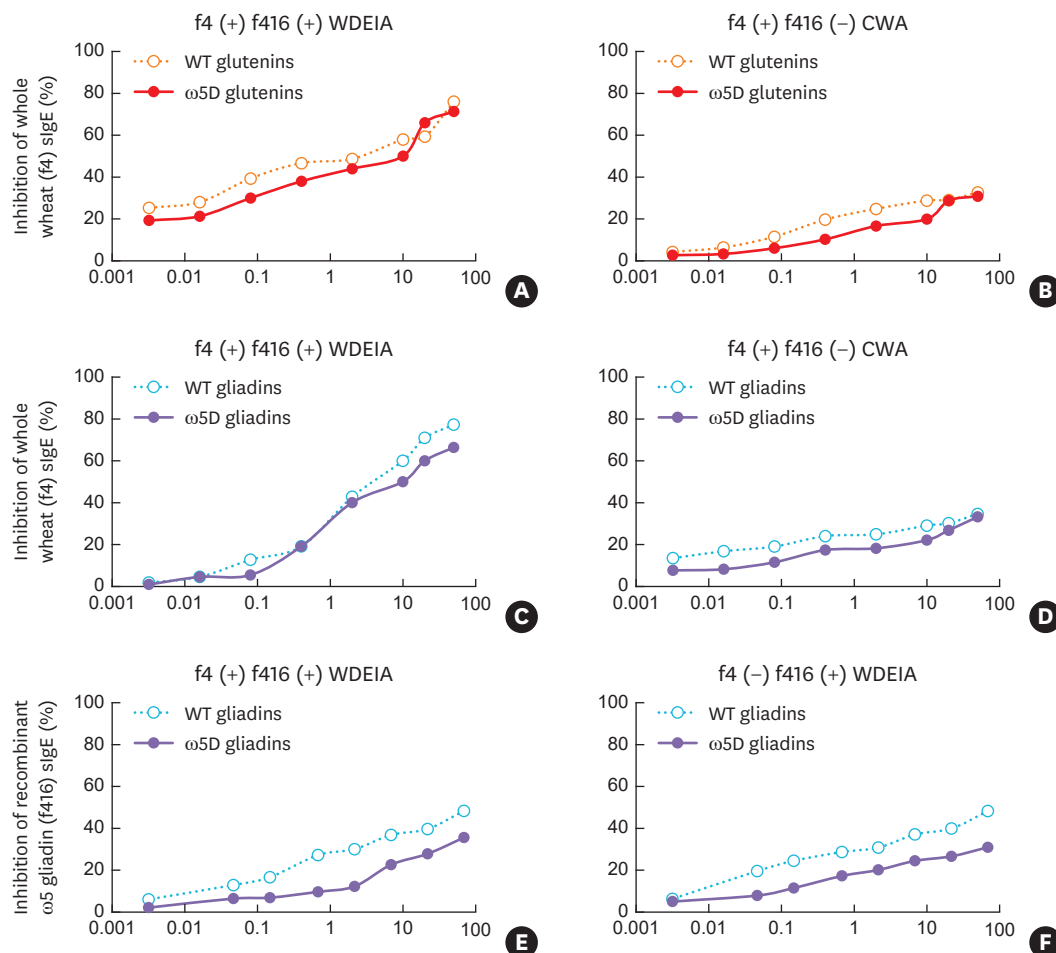

Fig. 6. ImmunoCAP inhibition assay for WT whole-wheat extract (A-D) and recombinant ω-5 gliadin (E, F) sIgE reactivity. Gliadins and glutenins from WT and ω5D cultivars were used as inhibitors. Pooled serum samples from WDEIA (A, C, E, F) and CWA (B, D) patients were used.
WT, wild-type; sIgE, specific immunoglobulin E; WDEIA, wheat-dependent exercise-induced anaphylaxis; CWA, classic wheat allergy.

inhibition produced by the gliadins isolated from the ω5D cultivar was approximately 35.6% compared to 48.4%, as produced by the gliadins isolated from the wild-type cultivar, in the whole water/salt-soluble wheat extract-sIgE (+) and recombinant ω-5 gliadin-sIgE (+) WDEIA patients (**Fig. 6E**). Similar results were also found in the whole water/salt-soluble wheat extract-sIgE (−) and recombinant ω-5 gliadin-sIgE (+) WDEIA patients (**Fig. 6F**).

DISCUSSION

In this study, we compared the allergenicity of the ω5D and wild-type cultivars (*T. aestivum* ssp. *aestivum* L) in WDEIA patients using the serum samples of WDEIA patients. Our SDS-PAGE and IgE immunoblot assay results showed attenuated sIgE binding to the glutenins and gliadins extracted from the ω5D compared to those from the wild-type cultivar in WDEIA patients. As expected, there was no difference in the allergenicity between the ω5D and wild-type cultivars for the CWA patients who exhibit allergies in response to water- and salt solution-soluble proteins rather than gluten. However, in addition to chromosome 1B, chromosomes 1A and 1D also contain loci for ω and γ gliadins. So, some LMW glutenin subunits,[13] substantial amounts of ω-5 or γ-gliadins, and other glutenins remain in this ω5D cultivar. Previous quantitative analysis using reverse phase high performance liquid chromatography showed that the amount of ω-5 gliadins in this ω5D cultivar is 0.92% of total gliadins compared to 3.05%–7.27% of the parent cultivars,[9] and IgE inhibition assays in this study shows that the allergenicity of the ω5D was only one-fourth of those of the wild-type cultivar in the WDEIA patients.

WDEIA is a wheat allergy with high exposure thresholds compared to other classic food allergies, including peanut, egg, or milk allergies.[14,15] WDEIA patients usually develop symptoms after ingesting more than 100 g of wheat products in addition to engagement in physical activities or taking aspirin.[16] One study that performed the gluten oral provocation test demonstrated that the median cumulative amounts of pure gluten to elicit a positive response in WDEIA patients were 48 g at rest and 24 g during exercise, respectively. The gluten content in wheat flour ranges from 8%–12%, and the described gluten amounts are equivalent to 240–480 g of wild-type wheat flour.[15] Since some gluten allergens are still present in the ω5D, allergic symptoms may be induced if ingested in high amounts by WDEIA patients. However, this ω5D may permit a safer range for consumption by WDEIA patients with mild to moderate sensitivity because the allergenicity of the ω5D is one-fourth of that of the wild-type cultivar.

The high exposure threshold of WDEIA may come from the physicochemical properties of glutens. Gluten proteins are poorly digested in the human gastrointestinal tract, partly due to their unique property of water/salt insolubility. They are also resistant to degradation by gastric acid and pancreatic and mucosal proteases[17]; therefore, they usually remain in the intestinal lumen. Gluten proteins can elicit allergic symptoms by infiltrating the intestinal mucosa to reach the systemic circulation, particularly under conditions with increased intestinal permeability, such as those induced by exercise or the consumption of aspirin.[16,18] Tanaka *et al.*[19] have indirectly shown that the ω-5 gliadin is absorbed relatively well from the gastrointestinal tract compared to other gliadins by measuring the serum concentrations of the gliadins in a mouse model during exercise or rest, thus supporting the ω-5 gliadin as the major allergen that causes WDEIA. These findings suggest that the subject's physical conditions may determine the absorption rate of gliadins, and then the enhanced serum concentration of ω-5 gliadin induces anaphylaxis in the previously sensitized subjects.

Another important issue of this study is the presence of cross-reactivity between gliadins and glutenins. The ω-5 gliadin has been recognized as the major allergen that causes WDEIA and immediate-type wheat allergy.[5,7,20] However, the HMW and LMW glutenin subunits as well as the α/β- and γ-gliadins are also involved in the pathogenesis of WDEIA.[1,4,6,7,10,21] Gluten proteins have distinct domains rich in glutamine and proline, and these characteristics support the presence of cross-reactivity among gluten proteins.[22] Battais et al.[23] have shown that the α/β, γ, ω2, and ω5 gliadins all contain an IgE epitope with the same consensus sequence of QQX1PX2QQ. Our IgE immunoblot analysis using WDEIA patient serum samples exhibited high reactivity against glutenins and gliadins. These findings support that several gliadins and glutenins are the culprit allergens that cause WDEIA. The unique IgE epitopes of the ω-5 gliadin and LMW glutenin subunits have been shown to have partial cross-reactivity.[10] Our ELISA inhibition assay demonstrated the presence of cross-reactivity between gliadins and glutenins. However, the allergenicity of gliadins was 14 times stronger than that of glutenins. These findings suggest that the gliadins are the representative allergens that cause WDEIA, thus supporting the findings of previous studies.[4,7,10,20]

Gluten-free wheat is available in the market; however, consumers should recognize that gluten is the fundamental protein that determines the viscosity and extensibility of dough. Most gluten-free wheat flours consist of rice, corn, or wheat starch, which do not naturally contain gluten. Therefore, the loss of a taste and texture similar to wheat is inevitable. It is already well known that gluten contents vary significantly according to the variations of the common wheat cultivar (T. aestivum L),[8] but the difference is not sufficient for safe consumption in WDEIA patients. To solve this dilemma, many investigators have tried to develop low-gluten wheat lines using genetic engineering technologies,[24-26] or by selecting cultivars that lack the ω-5 gliadins specifically.[27,28] Denery-Papini et al.[26] demonstrated the low allergenicity of wheat/rye cultivars with translocations at the Gli-B1 site using the small number of wheat allergy patients' serum samples. However, these cultivars have poor bread-making properties due to the presence of secalins originating from rye and now withdrawn from the market.[26] Other groups have taken a Gli-B1 locus deleted cultivars registered in National Bio-resource Project. The investigated cultivar has satellite deletions in chromosome 1BS-18 that result in only 20% of the amount of ω-5 gliadin present in the wild-type cultivar. They have also demonstrated the hypoallergenicity of this ω-5 gliadin-deficient cultivar using guinea pig and rat gluten food allergy models,[27,28] suggesting it as a safe alternative for individuals with wheat allergies. However, they still need to show hypoallergenicity using human samples and food challenge tests with WDEIA patients.

Our study has strong points compared to these previous studies. Rather than using genetic engineering, our ω5D cultivar was bred from double-haploid lines derived from two pre-existing wheat cultivars: the hard white Keumkang and soft red Olgeru cultivars.[9,11,12] So, there is no risk of gaining new allergenic epitopes in this ω5D cultivar. Although the quantity of ω-5 gliadins is about one-fourth of the parent cultivars, there was no difference in the percentages of the α/β, ω-1/2, and albumin between the two cultivars.[9] This exquisite difference in the composition of gliadins allows the ω5D to maintain the original rheology of wheat dough and palatable wheat products.[12] However, the presence of other gluten proteins in the ω5D cultivar may make it intolerable for patients with celiac disease, where the α/β-, ω-1/2, and γ-gliadins have been recognized as the major culprit factors.[17,29]

Our study also has limitations in evaluating allergenicity in WDEIA patients. The evaluation of sIgE reactivity may not be sufficient for a final interpretation of allergenicity. A double-

blind, randomized oral provocation test in WDEIA patients using wild-type and ω5D cultivars is required for a conclusion regarding the clinical practicality of this cultivar. We are now preparing for the randomized clinical trials. Finally, we enrolled a number of WDEIA and CWA patients with well standardized ImmunoCAP sIgE results to wheat whole water/salt-soluble extract and recombinant ω-5 gliadins, respectively. Although the clinical information strongly supports the diagnosis of WDEIA and CWA, provocation tests were absent except for one patient. So, the possibility of wheat sensitization without clinical significance could not be excluded.

Based on our findings, we suggest that the ω5D wheat cultivar may be a practical and safe alternative with a similar taste and texture to wild-type wheat for WDEIA patients. However, the presence of cross-reactivity between gliadins and glutenins as well as the presence of 1D chromosome-encoded gliadins in the ω5D wheat cultivar requires double-blind randomized oral provocation tests to prove the safety.

ACKNOWLEDGMENTS

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