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ORIGINAL RESEARCH

Omega-5 and Gamma Gliadin are the Major Allergens in Adult-Onset IgE-Mediated Wheat Allergy: Results from Thai Cohort with Oral Food Challenge

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Background: Various clinical patterns based on routes of sensitization and sensitized allergens are reported in adult-onset IgE-mediated wheat allergy. There is still a paucity of data on IgE-bound wheat allergen profiles in wheat challenge-proven adult-onset wheat allergic cases. Therefore, we aim to identify the major sensitized allergens in Thai adult-onset wheat allergic patients whose first symptom occurred after the age of 18 years despite previous tolerance.

Methods: This cross-sectional pilot study recruited patients from the Thai Adult-onset IgE-mediated Wheat Allergy Cohort (TAWAC). The sera of patients with mostly challenge-proven cases were selected for allergen study, including ImmunoCAP and IgE-bound gliadins along with glutenins profiles. The IgE-bound proteins were identified by liquid chromatography-tandem mass spectrophotometry (LC-MS/MS). Direct binding of IgE to recombinant gliadin and glutenin was performed to confirm the results of immunoblot and LC-MS/MS.

Results: Eleven wheat-dependent exercise-induced anaphylaxis (WDEIA) and 4 typical wheat allergy (WA) patients were enrolled. Serum IgE from >50% of bound proteins had a molecular weight ranging from 35 to 55 kDa in both gliadin and glutenin extracts. Further, ELISA demonstrated that γ-gliadin and ω5-gliadin were the most important major allergens. Other major allergens include α/β-gliadin, HMW glutenin, and possibly α-amylase inhibitor or LWM glutenin. Gamma-gliadin sensitization was found in all WA patients (4/4), while ω-5 gliadin was found in all WDEIA patients (11/11) from ELISA.

Conclusion: Wheat γ-gliadin and ω-5 gliadin are major wheat allergens among adult-onset wheat allergy patients in Thailand. Component-resolved diagnosis using γ-gliadin might be helpful in high suspicion of wheat allergy.

Keywords: allergens, anaphylaxis, enzyme-linked immunosorbent assay, food allergy, gliadins, gluten, immediate hypersensitivity, prolamin, wheat allergy

Introduction

Wheat proteins account for 10–15% of dry weight. They are classified based on the soluble property in a solvent. The two main groups of wheat allergens are the salt-soluble fraction, composed of the alpha-amylase/trypsin inhibitor subunit, and the salt-insoluble fraction, called the gluten fraction. The gluten fraction is composed of 2 main types of protein, monomeric and polymeric proteins. Monomeric proteins are classified into alpha-, beta-, γ-, and ω-gliadins whereas polymeric proteins, namely glutenins, consist of subunits of high molecular weight (HMW) and low

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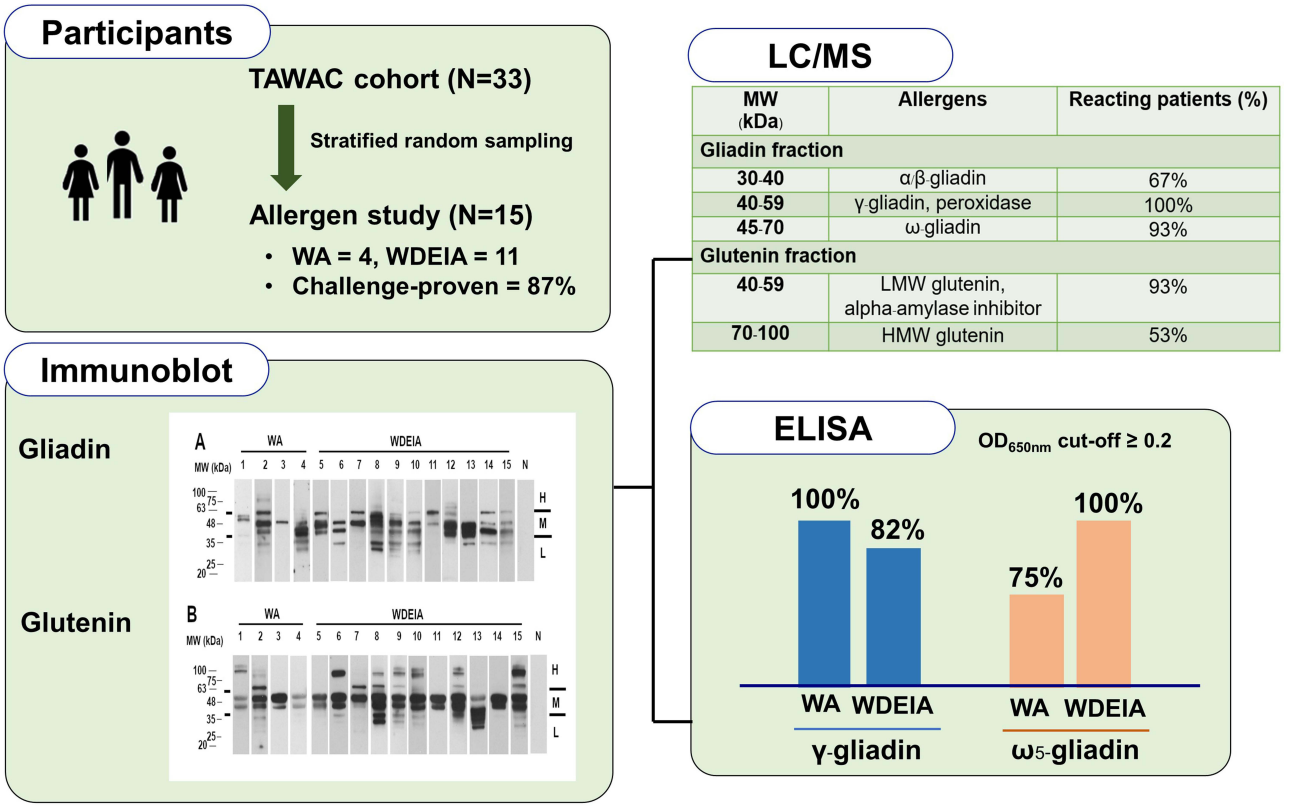
Graphical Abstract



molecular weight (LMW) peptides linked by disulfide bridges.[1] Wheat proteins are involved in 3 routes of sensitization including inhalation, skin contact, and ingestion, and the route involved in each case depends on the manner of the allergen exposure and immune mechanism.[2,3]

Wheat allergy is characterized by T helper 2 (TH2) activation which can result in immunoglobulin E (IgE) and non-IgE mediated reactions. IgE-mediated reactions are immediate, are characterized by the presence of wheat-specific IgE antibodies, and can be life-threatening.[4] Various clinical patterns according to routes of sensitization and sensitized allergens have been well described.[2] Adult-onset wheat allergy in which the first allergic symptoms occur after the age of 18 years despite previous tolerance has been reported in many countries.[5–7] A distinct phenotype called "wheat-dependent exercise-induced anaphylaxis (WDEIA)"[4] was reported to be common in adolescents and adults and was also found in the majority of patients in the TAWAC.[8] WDEIA could occur with the presence of cofactors other than exercises, such as pollen exposure, concomitant ingestions of non-steroidal anti-inflammatory drugs (NSAIDs) or alcohol, the presence of menses in females, infection, and stress.[9–11] The IgE response was diverse among the clinical phenotypes (ie, conventional wheat allergy vs WDEIA), geographic distribution, and unique clinical conditions (eg, hydrolysate wheat protein in Japanese adults).[12]

Few studies have addressed food allergens in oral food challenge (OFC)-confirmed cases, for which it is very important to confirm the food allergy in adults[13] and no studies have addressed allergen patterns in Thai adult-onset wheat allergic patients. In our cohort,[8] we reported a high diagnostic yield of the modified-3-day protocol to confirm the diagnosis of conventional wheat allergy (WA) and WDEIA. A similar protocol, using cofactors, was performed and yielded a similar result.[14] In the present study, we aimed to identify the important wheat allergens in adult-onset wheat allergic patients, primarily focusing on challenge-proven cases.

Methods

Subjects

Serum was prepared from blood samples of 15 adult-onset wheat-allergic patients from the TAWAC at the

Department of Medicine, Siriraj Hospital, Mahidol University, Thailand. The detailed information on the patients in TAWAC was reported in a previous publication.[8] In brief, the inclusion criteria for TAWAC were 1) an adult patient (age 18–60 years) with the onset of first wheat-allergic symptoms occurring after the age of 18 years, 2) a typical IgE-mediated reaction relating to wheat ingestion, 3) a temporal relationship with wheat ingestion, 4) positive allerologic workup with at least 1 of the following, skin tests, specific IgE to wheat allergens using ImmunoCAP with the condition that in the absence of SPTs and sIgE, the patients must have had at least 2 episodes of recurrence, the last reaction of which having occurred within 1 year of the time of recruitment. All participants with no food challenge contraindications were asked to volunteer for a wheat challenge. Two phenotypes were included in our study including 4 WA and 11 WDEIA patients. Baker's asthma/allergic patients were excluded. Sera from 5 healthy donors who had no history of food allergy and could tolerate wheat consumption was used as the control serum.

The study was approved by the Siriraj Institutional Review Board (SIRB), Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand (Approval code 600/2560, EC3). Written informed consent to use patient data and biological samples for medical research was done. This study was performed in accordance with the Declaration of Helsinki.

ImmunoCAP and Wheat Challenge

The specific IgE against whole wheat allergens, ω5-gliadin, lipid transfer proteins (LTPs) were measured by ImmunoCAP assays (Phadia, Uppsala, Sweden) with a lower limit of detection (LOD) of < 0.35 kUA/L, according to the manufacturer's recommendation. All patients with no contraindications to food challenge were asked to volunteer for wheat challenge. The 3-day modified wheat-cofactor challenge protocols were performed in 18 of 33 patients in our cohort as previously reported.[8] This protocol allowed for exercise-induced anaphylaxis (EIA), WA, and WDEIA to be diagnosed if a positive result occurred on days 1, 2, and 3, respectively. The contraindications of wheat challenges in our study included 1) having a history of severe anaphylaxis to wheat resulting in profound hypotension, neurological compromise, or respiratory failure and 2) having active cardio-neuro-pulmonary diseases, such as coronary heart disease, asthma, chronic obstructive lung disease, epilepsy, or psychiatric diseases.

Serum Collection for IgE-Allergen Profiles

Serum was collected from participants in our cohort during a visit to our center within 1 year after the most recent anaphylactic reaction to ensure that the demonstrated allergens correlated with the clinical reaction as the IgE pattern could have changed over time.

Extraction of Wheat Gliadins and Glutenins

Extracts of alcohol-soluble wheat gliadins and glutenins were prepared using a modified extraction protocol.[15,16] Briefly, 100 mg of raw wheat flour is mixed in 1 mL of 50 mM Tris-HCl pH 8, 0.5 M NaCl with continuous mixing for 1 h at room temperature (RT), followed by centrifugation at 17,210 × g for 10 min at 4 °C. This step is repeated before the pellet is mixed with 1 mL of buffer IT composed of 50% (v/v) aqueous isopropanol and 50 mM Tris–HCl pH 8 with continuous mixing for 30 min at RT, followed by centrifugation at 17,210 × g for 10 min at RT. The supernatant containing gliadins (gliadin extract) is collected before the pellet is extracted in buffer IP one more time. The pellet from the gliadin extraction is mixed with 50% (v/v) aqueous isopropanol, 50 mM Tris–HCl pH 8, 1% (w/v) dithiothreitol (DTT) and incubated at 60 °C with vigorous mixing every 5–10 min for 1 h, followed by centrifugation at 17,210 × g for 10 min at RT. The supernatant, or "glutenin extract" is collected. The concentration of proteins in both extracts is determined by bicinchoninic acid (BCA) protein assay.

Profiles of IgE-Bound Gliadins and Glutenins

For each serum collected from 15 patients, the serum was diluted at 1/10-1/200, based on ImmunoCAP results, in Phosphate Buffered Saline (PBS) (10 mM Na_2HPO_4, 2 mM KH_2PO_4, 2.7 mM KCl, 137 mM NaCl) containing 3% non-fat dry milk (buffer A) before use. As a control, the serum of 5 healthy, wheat tolerant donors was diluted at the same dilution as patients in the same experiment.

Immunoblot was performed. Per well of 12% SDS-PAGE gel at constant current, 20 µg of total proteins in the gliadin or glutenin extract was resolved. The two gels were run simultaneously. One gel was stained in a solution

containing Coomassie Brilliant Blue G250 for 1 h before submerging in distilled water. For other gels used in immunoblot, separated proteins in the gel were electro-transferred onto a nitrocellulose membrane. The membrane was cut into strips before incubating in buffer A for 1 h at RT and rinsing with PBS containing 0.2% v/v tween-20 (buffer B). One membrane strip was incubated with a diluted serum of one donor overnight at 4°C. The membrane strips were washed with buffer B before incubating with 1:10,000 diluted horseradish peroxidase (HRP) conjugated goat IgG anti-human IgE antibody (KPL, USA) in buffer A for 1 h. After washing, the membrane was incubated with the HRP substrate (Millipore, USA), and the emitted signal was captured by radiograph. The sera of control groups were diluted at the same dilution as the patients in the same experiment.

Identification of IgE-Bound Proteins in the Gliadin and Glutenin Extracts

The IgE-bound proteins in the gliadin and glutenin extracts were identified by Liquid chromatography-tandem mass spectrophotometry (LC-MS/MS). Briefly, by matched positions of resolved protein bands in SDS-PAGE gel with those of IgE bound proteins from the results of IgE profiles, gel pieces of matched IgE-bound proteins were excised from de-stained SDS-PAGE gel before submerging in 50 mM ammonium bicarbonate solution containing 50% acetonitrile (ACN) until colorless and then in 10 mM DTT for 15 min at 60 °C. The gel pieces were next submerged in 50 mM ammonium bicarbonate containing 55 mM iodoacetamide for 30 min at RT in darkness. The gel pieces were dried in 100% ACN before incubating in 50 mM ammonium bicarbonate containing 0.1 mg/mL trypsin (Sigma-Aldrich, USA) at 37°C overnight. The reaction was mixed with ACN at a 1:1 (v/v) ratio and incubated for 20 min. The solution was dried at 45°C before the peptides were resolved in 0.1% formic acid before injection into an Ultimate 3000 nano-LC system (Dionex, Surrey, UK) coupled with MicroToF Q II mass spectrometer (Bruker, Bremen, Germany). The mass spectra data were acquired using Hystar software (Bruker Daltonics, Germany) and were converted by Compass DataAnalysis software (Bruker Daltonics, Germany). The converted files were analyzed with the Mascot server version 2.6.2.1 (Matrix Science, USA) to search for matched sequences in the National Center for Biotechnology Information database with 95% confidence.

Direct Binding of Serum IgE to Recombinant Wheat Allergens

Recombinant γ-gliadin and ω5-gliadin were obtained from MyBioSource (CA, USA). Recombinant γ -gliadin was expressed in E.coli as 41.2 kDa mature protein (aa20-327, UniProt #P08453) with N-terminal 10x His-tagging, while recombinant ω5-gliadin was also expressed in E.coli as a 37.5 kDa truncated protein (aa262-439, UniProt #Q402I5) with N-terminal 6x-His-SUMO tagging. Both recombinant allergens were affinity-purified through the Immobilized Metal Affinity Chromatography (IMAC) column before sterile filtering. Both recombinant allergens were diluted into PBS and coated at 500 ng per well in a 96-well Maxisorp plate (Nunc, USA) and incubated at 4°C overnight. The coated well plate was washed with PBS-A (PBS + 3% non-fat dried milk). The serum of each patient and control was diluted 1/5 in PBS-A. Diluted serum was incubated with coated allergens in PBS-A for 2 h at RT. The plate was washed before adding diluted HRP-labelled goat IgG anti-human IgE antibodies into the designated well. Substrate 3,3′,5,5′-tetramethylbenzidine (TMB) (Thermo Fisher, USA) was added to each well before absorbance at $OD_{650\ nm}$ was measured. The negative controls were HRP-labeled goat IgG anti-human IgE binding to coated allergens, buffer solution incubated with coated allergens, and the control serum which was diluted the same as the serum of patients in the same experiment.

Statistical Analysis

All analyses were performed using PASW Statistics version 18.0 (SPSS, Inc., Chicago, IL, USA). Demographic and clinical data were summarized using descriptive statistics. Categorical data are presented as frequency (percentage). Continuous data are presented as median (range). Student *t* test was used for the comparison of normally distributed continuous variables, Mann–Whitney *U*-test for the comparison of nonnormally distributed continuous variables, and the chi-square test or Fisher's exact test for comparing categorical variables between the 2 groups.

Results

Patients

Figure 1 summarizes the enrollment of participants in this study. A total of 33 participants met the inclusion and were included in TAWAC. We primarily focus on challenge-proven cases. Stratified random sampling was performed with a ratio of typical wheat allergy (WA): wheat-

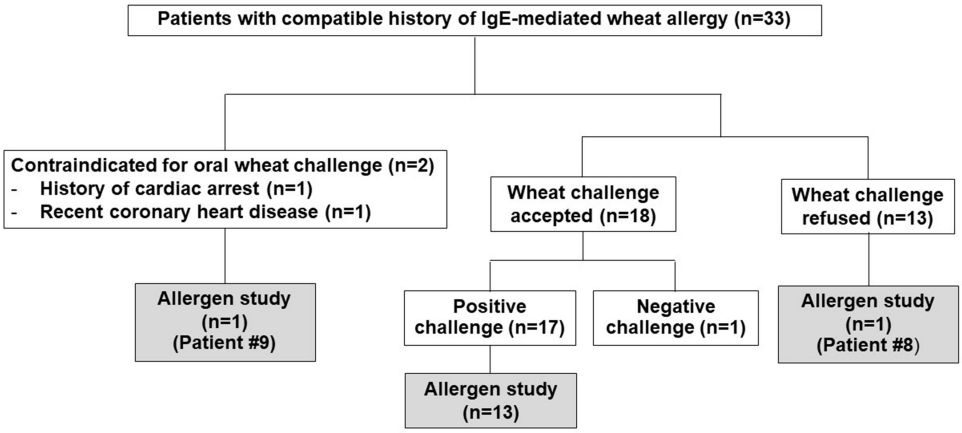


Figure 1 Patient recruitment in the study.
Notes: Patient #8 refused oral wheat challenge, and patient#9 had a contraindication for oral wheat challenge (recent coronary heart disease).

dependent exercise-induced anaphylaxis (WDEIA) = 1:2. Additional 2 patients were selected from the challenge-refuse group and challenge-contraindicated group. The baseline characteristics of 15 patients (enrolled in this allergen study) and all 33 patients in TAWAC were similar (Table 1). A total of 15 participants were included for allergen analysis including 13 participants with a positive challenge along with 1 patient who refused wheat

Table 1 Baseline Characteristics of the 15 Patients Enrolled in the Present Study and All 33 Patients in TAWAC Cohort

	Enrolled Patients in the Current Study (N=15)	All Patients in the TAWAC Cohort (N=33)	*p*-value
Gender Male Female	 2 (13.3) 13 (86.7)	 9 (27.3) 24 (72.7)	0.46
Age, mean ± SD, year	32.3 ± 11.6	32.6 ± 11.3	0.93
Age of onset, ± SD, year	29.9 ±10.3	29.7 ± 10.5	0.97
Wheat allergy phenotype [a] WDEIA WA	 11 (73.3) 4 (26.7)	 23 (69.7) 10 (30.3)	 1.00 0.51
Duration of disease, year	1.0 (1.0–5) (min= 0.50, max= 8.0)	2 (1.00–4.00) (min= 0.25, max= 17)	0.857
Number of episode before recruitment	4(2–6)	4(2–6)	0.640
Anaphylaxis severity [a] Grade 1 Grade 2 Grade 3 Grade 4	 0 (0) 8(53.3) 7(46.7) 0 (0)	 2 (6.1) 16 (48.5) 14 (42.4) 1 (3.0)	1.00
sIgE to wheat[b] level, kUA/L	1.18 (0.58–3.03)	0.54 (0.28–1.53)	0.257
sIgE to ω-5 gliadin[b] level, kUA/L	5.84 (1.03–9.48)	3.99 (1.28–9.06)	0.920
Total IgE (IU/mL), IU/mL	400 (68.5–1160)	211 (100–508)	0.430

Notes: Number of patients (% within group), mean±SD and median(P25-P75). [a] Anaphylaxis severity: Ring and Messmer: grade 1–4. [b] Allergen-specific IgE using solid-phase immunoassay, ImmunoCAP (Phadia, Uppsala, Sweden).
Abbreviations: IU/mL, international units per milliliter; kUA/L, kilounits of allergen-specific IgE per liter; nsLTP, non-specific lipid transfer protein; SD, standard deviation; sIgE, specific immunoglobulin E; TAWAC, Thai Adult-onset IgE-mediated Wheat Allergy Cohort; WA, conventional wheat allergy; WDEIA, wheat-dependent exercise-induced anaphylaxis.

challenge (patient #8) and 1 patient with OFC contraindication (patient #9). Patients #8 and #9 both had at least 2 episodes of wheat-related anaphylaxis, which increases the probability that they had genuine wheat allergies.

Table 2 summarizes the detailed clinical characteristics of 15 patients enrolled in the allergen study. The median age of wheat allergy onset was 30 years (range, 18–48). Female was more common than male (13 vs 2). There were 11 WDEIA patients and 4 WA patients. According to the clinical criteria from the National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network Symposium (NIAID/FAAN),[17] all patients had experienced anaphylactic episodes.

Identification of Wheat Allergens

ImmunoCAP

Using the LOD of >0.35 kUA/L as the positive cut-off point, 12 patients (80%) and 13 patients (89.7%) had positive results for wheat and ω-5 gliadin-specific IgE, respectively. The median level of specific IgE to wheat and ω-5 gliadins was 1.18 (range, 0.06–35.4) and 5.84 kUA/L (range, 0.07–43.8), respectively. Only 1 patient sensitized to non-specific (ns-) LTP at low sIgE level (0.55 kUA/L). The median total IgE level was 400 IU/mL (range, 24.6–3,420) at the same time point when collecting blood samples for specific IgE measurements.

Profiles of IgE Bound Gliadins and Glutenins and LC-MS/MS Analysis

The profiles of IgE-bound gliadins and glutenins of all 15 patients (4 WA and 11 WDEIA) are demonstrated in Figure 2. The profiles of all patients showed serum IgE-bound 40–59 kDa gliadins and glutenins. The analysis of LC-MS/MS results (Table 3) showed 40–59 kDa gliadins were γ-gliadin, peroxidase, and ω-gliadin (M, H in Figure 2A), while 40–59 kDa glutenins were LMW glutenin and alpha-amylase/subtilisin inhibitor (M in Figure 2B). Besides the 40–59 kDa gliadins and glutenins, >50% of WA and WDEIA patients had serum IgE-bound 30–40 kDa α/β-gliadin (L in Figure 2A) and 70–100 kDa HMW glutenin (H in Figure 2B).

Direct Binding Enzyme-Linked Immunosorbent Assay

The enzyme-linked immunosorbent assay (ELISA) reflects direct IgE binding to specific allergens, including γ and ω-5 gliadin. The result is demonstrated in Figure 3. The details of the ELISA result are shown in Table 4. ELISA demonstrated that 100% of WA and 82% of WDEIA patients sensitized to γ-gliadin, 75% of WA and 100% of WDEIA patients sensitized to ω5-gliadin.

Because an OD value of negative controls (HRP-labeled goat IgG anti-human IgE binding to coated allergens and buffer solution incubated with coated allergens) was 0.06 from multiple and separated ELISA assays, we use >3 fold-value of the negative controls which would be 0.2 as a cut-off point. Using the cut-off value of $OD_{650\ nm} \geq 0.2$, 13 patients (86.7%) were sensitized to γ-gliadin with 2 patients having a marginal level of 0.2. Fourteen patients (93.3%) were sensitized to ω5-gliadin. Patient#4 was negative for sIgE to ω5-gliadin (0.23 kUA/L) and marginally above the cut-off for ELISA IgE-bound ω5 gliadin ($OD_{650\ nm}$= 0.22). This patient demonstrated a high titer of sIgE to wheat (33.4 kUA/L), and it was above the cut-off for ELISA IgE-bound γ gliadin ($OD_{650\ nm}$= 0.49). Patient #15 was excluded from ELISA due to a low-value sIgE for both wheat and ω5-gliadin.

Discussion

This is the first study investigating the IgE sensitization pattern in Thai adult-onset wheat allergic patients, most of whom were challenge-proven cases (86.7%). We have identified that the important wheat allergens for WA are ω5-gliadin, γ-gliadin, peroxidase, ω1,2-gliadin, and LMW glutenin. Overall, the results of ELISA demonstrated that 86.7% and 93.3% of patients were sensitized to γ-gliadin and ω5-gliadin, respectively.

Since several wheat proteins appear naturally as a complex mixture of components with inter- and/or intra-chains,[1] and the bands of IgE-bound proteins from both gliadin and glutenin extracts were excised from 1-dimension SDS-PAGE gel, multiple gliadins and glutenins were identified as shown in the LC-MS/MS results. To confirm the LC-MS/MS results, direct IgE binding to recombinant wheat proteins confirmed that both γ- and ω5- gliadin are major wheat allergens, and >80% of all patients in our study had serum IgE against them. The results suggest that determining serum IgE to γ- and ω5-gliadin may increase the diagnostic accuracy in suspected adult-onset wheat allergy patients. Other glutenins, LMW- and HMW-glutenin, may also need to be determined as IgE markers for adult-onset wheat allergy.

Although recombinant ω5-gliadin could identify patients who have positive ω5-gliadin sIgE, the results of IgE-binding ELISA and ImmunoCAP of some patients may show discordant binding values as some IgE epitopes may not be in the truncated recombinant ω5-gliadin

Table 2 Clinical Characteristics of the Patients in the Allergen Study (n=15)

Patient	Sex/Age of Onset (Years)	Diagnosis After Wheat-Cofactor Challenge	Symptoms According to History	CAP[a] sIgE Wheat (kUA/L)	CAP[a] sIgE ω5-Gliadin (kUA/L)	CAP[a] sIgE ns-LTP (kUA/L)	Total IgE (IU/mL)	Grass Pollen Sensitization[b]
1	F/20	WA	U, A, D, GI	0.1	0.93	0.01	99.7	Ber
2	F/35	WA	U, A, D	1.42	7.44	0.12	3420	Ber
3	F/37	WA	U, A, D, BP	1.18	5.84	0.55	3340	Ber
4	F/18	WA	U, A, GI	35.4	0.23	0.14	1160	Ber
5	F/45	WDEIA	U, A, D, GI	3.03	24.2	0.04	1350	No
6	F/27	WDEIA	U, Syncope, BP	2.69	9.48	0.04	587	Ber, John
7	F/20	WDEIA	U, D	1.06	6.15	0.05	353	No
8[c]	F/33	Probable WDEIA	U, A, D, Syncope	0.68	7.85	0.12	545	No
9[c]	F/37	Probable WDEIA	U, A, D, Syncope, GI	0.58	1.28	0.04	53.9	No
10	F/19	WDEIA	U, D	1.24	1.03	0.08	195	Ber
11	F/18	WDEIA	U, A, D	10.9	43.8	0.08	640	Ber, John
12	M/30	WDEIA	U, Syncope	0.09	2.75	0	59.6	No
13	F/21	WDEIA	U, D, GI	0.22	1.84	0	24.6	No
14	F/40	WDEIA	U, D, Syncope, BP	1.09	15.2	0.01	400	No
15	M/48	WDEIA	U, D, Syncope	0.06	0.07	0	68.5	No

Notes: [a]Allergen-specific IgE using solid-phase immunoassay: ImmunoCAP (Phadia AB, Uppsala, Sweden). A positive test result is defined by a level of allergen-specific IgE ≥ 0.35 kUA/L [b] Demonstrated by skin prick test. Histamine (10 mg/mL) and normal saline were used as negative and positive controls. A positive test result is defined as a wheal diameter >3 mm larger than the negative control. [c] Did not undergo wheat-cofactor challenge due to refusing challenge (Patient#8) and severe reaction with cardiovascular comorbidity (Patient#9).

Abbreviations: Ber, bermuda grass; BP, hypotension; D, dyspnea; GI, gastrointestinal; IgE. immunoglobulin E; IU/mL, international units per milliliter; John, Johnson grass; kUA/L, kilounits of allergen-specific IgE per liter; nsLTP, non-specific lipid transfer protein; SD, standard deviation; SPT, skin prick test; sIgE, specific immunoglobulin E; U, urticarial; WA, conventional wheat allergy; WDEIA, wheat-dependent exercise-induced anaphylaxis.

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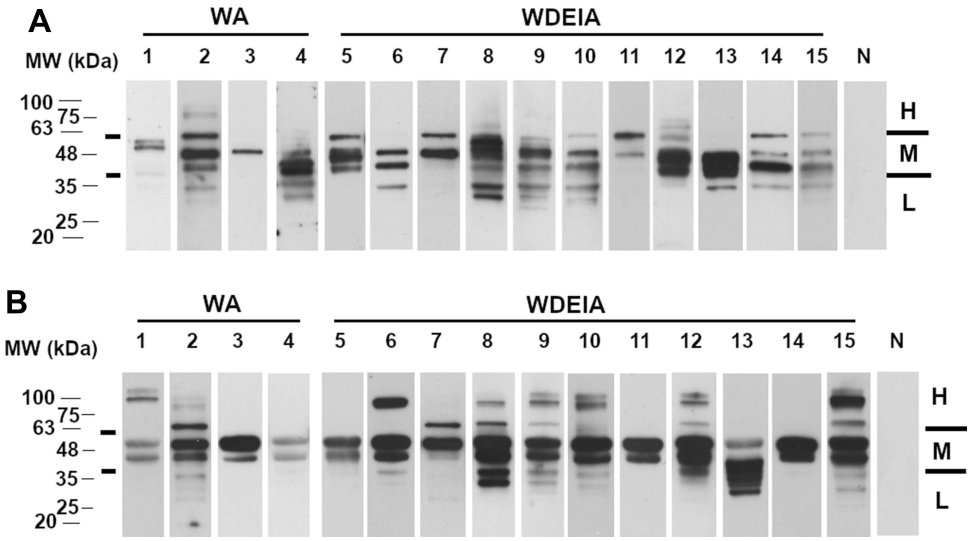

Figure 2 IgE immunoblotting of gliadin **(A)** and glutenin **(B)** extracts.
Notes: L = proteins in MW range of <40 kDa; M = proteins in MW range of 40–59 kDa; H = proteins in MW range of >60 kDa.
Abbreviations: WA, Wheat allergy group (patients 1–4); WDEIA, Wheat-dependent exercise-induced anaphylaxis group (patients 5–15); MW, molecular weight marker; kDa, kilodaltons.

(aa262-439). In the present study, only 1 patient (Patient#1 with WA) had an IgE-bound ω5-gliadin OD of 0.18 but had an IgE-bound γ -gliadin OD of 0.52 from ELISA. The level of IgE-bound $OD_{650\ nm}$ indicates that each individual has a different level of sIgE to certain allergens. In contrast, sIgE to crude wheat extract in ImmunoCAP could only imprecisely indicate the presence of sIgE to some of the multiple wheat allergens. When considering the results of ELISA and ImmunoCAP, there was a proportion of patients who were sensitized to γ-gliadin. Therefore, the diagnosis of adult-onset wheat allergy using these 2 single allergens could provide more information.

Table 3 Identification of IgE-Bound Gliadin and Glutenin

Immunoblot	Molecular Mass (kDa)	Protein
Gliadin fraction		
L	30–40	α/β-gliadin
M	40–59	γ-gliadin, peroxidase
M, H	45–70	ω-gliadin
Glutenin fraction		
L, M	40–59	LMW glutenin, alpha-amylase/ subtilisin inhibitor
H	70–100	HMW glutenin

Notes: L = proteins in MW range of <40 kDa; MW = Molecular weight; M = proteins in MW range of 40–59 kDa; H = proteins in MW range of >60 kDa.
Abbreviations; kDA, kilodaltons; LMW, low molecular weight; HMW, high molecular weight.

There have been significant advances in the description of many allergens causing IgE-mediated wheat allergies. ImmunoCAP is widely used for allergen-specific IgE measurements, which could quantify allergen-specific IgE antibody levels. However, ImmunoCAP allergen extracts are limited to the composition of the extract. To date, the available wheat allergen-specific IgEs are whole-wheat extract and allergen components such as ω5-gliadin (Tri a 19), LTPs (Tri a 14), and gliadins.[2] None have reached an acceptably high specificity and sensitivity to become a reference standard for diagnosis, especially in adult wheat allergic patients.

Previous data from Australian WDEIA patients demonstrated ω5-gliadin sIgE had a sensitivity of 91% and specificity of 92% for wheat allergy diagnosis.[18] Data from our previous study on adult-onset wheat allergy demonstrated a positivity rate of specific IgE (cut-off, > 0.35 kUA/L) for wheat and ω5-gliadin were 61% and 88%, respectively.[8] The definite diagnosis still relies on OFC done under medical supervision. However, the WDEIA phenotype in adult wheat allergy might have negative results with conventional OFC.[4] Combining OFC with cofactors could increase the diagnostic yield. Nevertheless, the procedure cannot be performed in all cases and should be used cautiously as severe reactions can occur.[8,10,11,14]

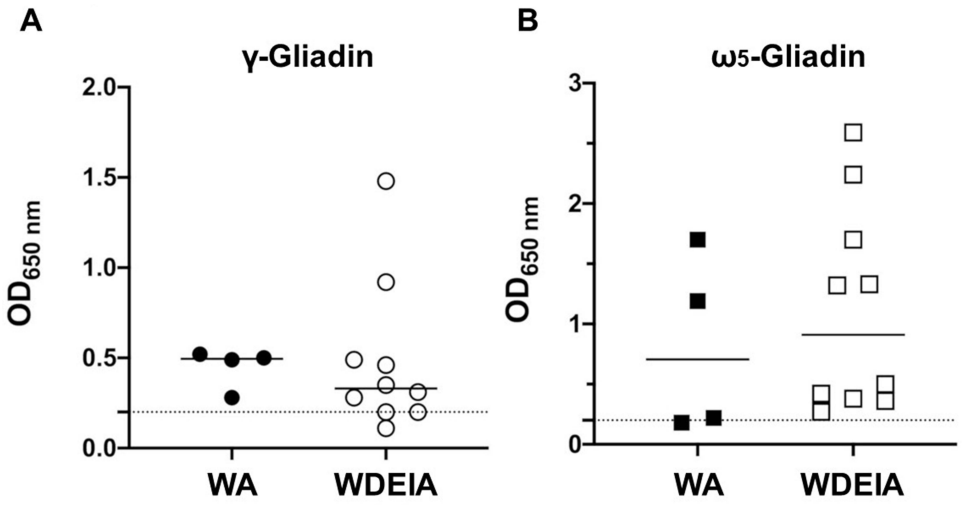

Figure 3 Direct binding ELISA. (**A**) γ-gliadin, (**B**) ω5-gliadin.
Notes: The dotted-line denotes a cut-off value. The median is shown for each group. The solid line denoted mean $OD_{650\ nm}$ level within the group. The serum of one wheat-dependent exercise-induced anaphylactic patient (patient #15) was excluded due to a low value of specific IgE to both wheat and ω5-gliadin.
Abbreviations: ELISA, enzyme-linked immunosorbent assay; OD, optical density; nm, nanometer.

Because adult-onset wheat allergy has various clinical patterns according to routes of sensitization and sensitized allergens,[2] the IgE response could be diverse among the clinical phenotypes, geographical distribution, and unique clinical conditions.[12] Therefore, the identification of sensitized allergen is the pivotal first step towards appropriate component-resolved diagnosis (CRD) testing in any specific population. Based on our result, γ-gliadin could be helpful in a case with high suspicion of adult-onset wheat allergy, especially in patients who were negative sIgE to other wheat allergen components.

Our results differ from a multi-centered European study[13] in wheat-challenge-positive patients that demonstrated α-amylase/trypsin inhibitor were the most important wheat allergens. LTP in the albumin/globulin fraction and LWM glutenin in the gluten fractions were also important allergens. Although LTP was reported to be a major allergen in Italian patients,[13] LTP sensitization was rare in our cohort. Hofmann et al[19] reported

Table 4 ELISA Results of γ-Gliadin and ω5-Gliadin Compared with ImmunoCAP Results

Patients	IgE Bound γ-Gliadin (Mean $OD_{650\ nm}$)	IgE Bound ω5-Gliadin (Mean $OD_{650\ nm}$)	SIgE ω5-Gliadin ImmunoCAP (kUA/L)	SIgE Wheat ImmunoCAP (kUA/L)
1	0.52	0.18[a]	0.93	0.1
2	0.28	1.19	7.44	1.42
3	0.50	1.70	5.84	1.18
4	0.49	0.22	0.23	33.4
5	1.48	2.24	24.2	3.03
6	0.92	1.33	9.48	2.69
7	0.28	1.70	6.15	1.06
8	0.35	0.50	7.85	0.68
9	0.20	0.27	1.28	0.58
10	0.11[a]	0.38	1.03	1.24
11	0.49	2.59	43.8	10.9
12	0.20	0.42	2.75	0.09
13	0.46	0.36	1.84	0.22
14	0.31	1.32	15.2	1.09
15	0.19[a]	0.29	0.07	0.06

Note: [a] OD_{650} nm level < 0.2 cut-off.
Abbreviations: mean $OD_{650\ nm}$, the mean of optical densities at 650 nanometers; ELISA, enzyme-linked immunosorbent assay; kUA/L, kilounits of allergen-specific IgE per liter.

IgE profiles of 17 confirmed WDEIA patients using ImmunoCAP and microarray immunoassay. Using ImmunoCAP, IgE to ω5-gliadin was detected in 82% and IgE to α/β/γ-gliadin was detected in 82% including the 3 patients lacking IgE to ω5-gliadin. The microarray revealed that γ-gliadin was the second most important allergen. Our study supports their findings and suggests the additional diagnostic value of γ-gliadin. In Japan, Yagami et al reported an outbreak of immediate-type wheat allergy caused by hydrolysate wheat protein (HWP)-containing facial soap. Immunoblot analysis and ELISA revealed a distinct pattern from convention wheat allergy.[20] HWP or GP19S was acid-hydrolyzed wheat proteins processed through acid hydrolysis, which could induce different protein conformation, resulting in different IgE binding patterns from the ones in the present study[21,22] The Japanese population had a distinct feature that was a remission rate of 56.1% at 60 months after stopping the soap.[23] Recent consensus proposed 4 different clinical patterns of wheat allergy that could directly reflect the involved wheat allergens[24] Although there is generally a significant overlap of the responses to individual proteins in different wheat allergic phenotype conditions,[2] it is possible that the main route of sensitization in our cohort was gastrointestinal uptake as the most prevalent wheat allergens found in our study were gliadins.

Our explanatory study describes a pattern of allergen component sensitization in our cohort. We hypothesize that testing for γ-gliadin after negative for wheat and ω5-gliadin might have value in Thai adult wheat allergic patients. A similar finding was also supported by Hofmann et al.[19] Either of the replacement test or add-on test, using γ-gliadin, would need confirmation in a hypothesis-testing study, at least a prospective cohort study, and finally in a diagnostic randomized trial to test the outcomes of interest, including cost-effectiveness.

Our study has limitations. Although 2 patients did not undergo OFC due to OFC refusal (Patient#8) and recent coronary heart disease (Patient#9), both patients fulfilled additional criteria of repeated reaction that would increase the likelihood of genuine wheat allergy. The present study was also focused only on IgE-bound alcohol-soluble allergens, gliadins, and glutenins since these allergens are reported as major allergens in many regions.

Conclusion

Wheat γ -gliadin and ω-5 gliadin are major wheat allergens among adult-onset wheat allergy patients in Thailand. Component-resolved diagnosis using γ-gliadin might be helpful in high suspicion of wheat allergy.

Abbreviations

ELISA, enzyme-linked immunosorbent assay; GI tract, gastrointestinal tract; HMW, high molecular weight; HWP, hydrolyzed wheat protein; LMW, low molecular weight; NSAIDs, non-steroidal anti-inflammatory drugs; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SPT, skin prick test; sIgE, specific immunoglobulin E; WA, wheat allergy; WDEIA, wheat-dependent exercise-induced anaphylaxis.

Ethical Approval

This study was approved by the Siriraj Institutional Review Board and Ethics Committee of the Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand, approval no.600/2560 (EC3). (Date of approval: November 29, 2017). All patients provided informed consent to participate and for the data to be published.

Consent for Publication

All patients provided informed consent to participate and for the data to be published. The patient was informed that de-identified data would be used in scientific research and publications.

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Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval for the version to be published; and agreed to be accountable for all aspects of the work.

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Disclosure

All authors declare no personal, professional, or other conflicts of interest for this work.

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