IgE reactivity to carbohydrate moieties of glycoproteins in wheat allergy

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ABSTRACT

Carbohydrate moieties of different glycoproteins, such as cross-reactive carbohydrate determinants (CCDs) and galactose α-1,3-galactose, can induce IgE reactivity with varied clinical significance. In this study, the possible participation of glycan from wheat gliadin, with respect to its IgE-binding capacity, was investigated in children with food allergies to wheat. Total IgE and wheat-specific IgE quantification, documentation of history, and/or oral food challenge (OFC) were performed for 52 children. Subjects with positive wheat-specific IgE were characterized as the symptomatic group, never-exposed group, or asymptomatic group. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and glycan detection in gliadin were performed. IgE binding to gliadin and deglycosylated gliadin was measured by immunoblotting and ELISA. Gliadin-specific IgE was detected and correlated with wheat-specific IgE in the symptomatic, never-exposed, and asymptomatic groups. The glycan range overlapped significantly with the gliadin range. Deglycosylation of gliadin reduced the allergenicity of gliadin. In gliadin, the allergenicity of the glycan portion was greater in the symptomatic group than in the never-exposed and asymptomatic groups. We conclude that N-glycan in gliadin might exhibit allergenicity as a possible carbohydrate epitope in wheat allergy in children.

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Wheat is one of the most common foods causing food allergies in children.[1,2] Wheat proteins are classified based on their extraction in different solvents and include albumin, globulin, gliadin, and glutenin.[2] Water- and dilute salt-soluble fractions of wheat (albumins and globulins) comprise only 15%–20% of the total protein; most of the protein consists of the ethanol- and dilute acid-soluble wheat fractions (gliadins and glutenins).[3,4] Gliadins are grouped into three types, α/β-, γ-, and ω-gliadins.[5] Recently, specific IgEs to αβ-, γ-, and ω-gliadin were detected in Baker's asthma,[4,6,7] to γ-, and ω-gliadin in wheat-dependent, exercise-induced anaphylaxis,[8–10] and to gluten (which contains approximately equal amounts of gliadin and glutenin) in recurrent urticaria.[11] For patients with food allergies to wheat, specific IgEs to all wheat fractions were detected at various levels.[12–16]

A glycan is the carbohydrate portion of a glycoconjugate, such as a glycoprotein, glycolipid, or a proteoglycan.[17] Many or most of the allergens that we inhale or ingest are glycosylated with oligosaccharides.[2,18] Despite this, current evidence shows that the IgE antibodies associated with allergic disease are specific for protein epitopes, and most research has focused on protein epitopes.[18]

The presence of IgE antibodies to carbohydrate antigens was first identified from *in vitro* experiments looking at cross-reactivity between different plant-derived antigens.[19] In part because of this approach, the carbohydrate epitopes identified were generally, or exclusively, cross-reactive, which led to the designation cross-reactive carbohydrate determinants (CCDs).[18] It is well known that the carbohydrate moieties present in many plant foods can induce antiglycan IgE responses. However, the clinical significance of these CCDs is unclear.[19,20] By contrast, recent work has shown that IgE antibodies specific for the carbohydrate galactose-α-1,3-galactose are capable of eliciting serious, even fatal, reactions.[21,22]

In this study, we first aimed to evaluate the allergenicity and glycosylation of gliadin in wheat allergy. Subsequently, we investigated the possible participation of the glycan from gliadin of wheat in terms of its IgE-binding capacity in the sera of children with wheat allergy.
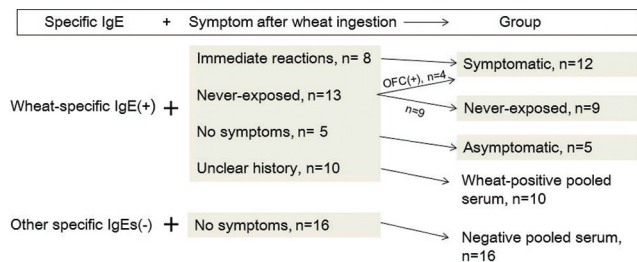
Figure 1. *Flowchart of study population.*

MATERIALS AND METHODS

Patients and Sera

Sera were obtained from 52 patients who visited the allergy clinic for food allergies or other allergic diseases at the Severance Hospital of Yonsei University and Ilsan Paik Hospital of Inje University. After venous blood was drawn, serum was separated and stored at −20°C until analysis.

In this study, wheat allergy was defined as IgE-mediated allergy to wheat based on case history, wheat-specific IgE concentrations, and/or oral food challenge (OFC). After wheat ingestion, the clinical reaction history of patients with positive wheat-specific IgE was categorized into four types: immediate reaction, no symptoms, never exposed, or unclear symptoms (Fig. 1). For patients with low wheat-specific IgE under the predictive diagnostic decision point (26 kU$_A$/L)[23] and no recent reactions to wheat, OFC was strongly recommended to assess the status of tolerance. For other patients, we could not perform OFC for various reasons, including recent reactions to wheat, severe atopic dermatitis, young age, parents' refusal, or no previous reactions to wheat despite the presence of high wheat-specific IgE levels.

We divided children who were positive for wheat-specific IgE and had a clear history about wheat allergy into three groups according to clinical history and/or OFC: the symptomatic group, the never-exposed group, and the asymptomatic group (Fig. 1).

Nine patients with high wheat-specific IgE who had never consumed wheat due to severe atopic dermatitis were grouped as the never-exposed group (Table 1) and five patients who had no symptoms after wheat ingestion despite high wheat-specific IgE were grouped as the asymptomatic group (Table 2).

Eight patients who had a clinical history of immediate hypersensitivity reactions after wheat ingestion with high wheat-specific IgE above the predictive diagnostic decision point, and four patients who showed positive results to OFC with wheat, were grouped as the symptomatic group (Table 3).

The sera of 10 patients who had high wheat-specific IgE levels and unclear histories of wheat allergies were used to create the wheat-positive pooled serum. These patients had severe multiple food allergies and severe atopic dermatitis. Therefore, their symptoms could not be unambiguously defined as a wheat allergy. The sera of 16 children who visited the clinic for asthma, allergic rhinitis, atopic dermatitis, or chronic cough without food allergies were used as negative pooled serum. These samples were negative for specific IgEs to all the evaluated allergens.

The Severance Hospital Institutional Review Board (4-2013-0317) and the Ilsan Paik Hospital Institutional Review Board (IB-2-1308-038) approved this study, and informed consent was obtained from the parents of the children.

ImmunoCAP Test for Total and Specific IgE to Wheat

Total and wheat-specific IgE levels were measured using the ImmunoCAP test (Pharmacia, Diagnostics, Uppsala, Sweden). For those patients whose sera were used to create the negative pooled serum, levels of specific IgE to *Alternaria*, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, pollen, or other foods according to their clinical history were measured instead of wheat-specific IgE. According to the manufacturer's instructions, specific IgE levels of over 0.35 kU$_A$/L were considered positive.

Open OFCs to Wheat

OFC to wheat was performed as an open challenge in a hospital setting and supervised by a physician in accordance with the guidelines of the Korean Academy of Pediatric Allergy and Respiratory Diseases.[24] Patients were challenged with boiled wheat noodles. The total dose of wheat noodles for OFC was adjusted according to age (90–200g). The test was started by consuming one strand of boiled wheat noodles (1.353 g), and this amount was doubled every 15 minutes until symptoms arose or until the entire test meal was consumed.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) of Gliadin

Gliadin (Sigma-Aldrich, St. Louis, MO) was purchased commercially. Gliadin (10 μg) was dissolved in sample buffer (60mM Tris-HCl, 25% glycerol, 2% SDS, 14.4mM 2-mercaptoethanol, and 0.1% bromophenol blue) and boiled for eight minutes. The denatured gliadin solution was then loaded and separated on 4%–20% gradient PAGE gels (Mini-PROTEAN TGX Gel; Bio-Rad, CA), and protein bands were stained with Coomassie blue. For immunoblotting, separated proteins were transferred onto polyvinylidene difluoride membranes (Immobilon P; Millipore Co., Billerica, MA).

Table 1. Characteristics of the never-exposed group (_n_ = 9)

Number	Age (years)	Sex	Diagnosis	Total IgE (IU/mL)	Wheat IgE (kU$_A$/L)	Clinical Symptoms After Ingestion of Wheat
1	0.6	M	AD, FA	>5000	>100	Never exposed
2	1	M	AD, FA	>5000	>100	Never exposed
3	0.92	M	AD	>5000	82.1	Never exposed
4	1.75	M	AD	>5000	>100	Never exposed
5	0.75	M	AD, FA	773	48.8	Never exposed
6	0.65	M	AD	5000	30.4	Never exposed
7	0.53	M	AD, AR	1488	73.5	Never exposed
8	2.1	M	AD, AR,	3190	38.8	Never exposed
9	2	F	AD, FA	>5000	>100	Never exposed

AD = atopic dermatitis; AR = allergic rhinitis; FA = food allergy.

Table 2. Characteristics of the asymptomatic group (_n_ = 5)

Number	Age (years)	Sex	Diagnosis	Total IgE (IU/mL)	Wheat IgE (kU$_A$/L)	Clinical Symptoms After Ingestion of Wheat
10	2.1	F	AD, FA	515	73.5	No symptoms
11	0.66	F	AD	798	22.4	No symptoms
12	5	M	AD, FA	501	22.6	No symptoms
13	2.1	M	AD, FA	3757	43	No symptoms
14	2.1	M	AD, FA	3606	46.7	No symptoms

AD = atopic dermatitis; FA = food allergy.

Table 3. Characteristics of the symptomatic group (_n_ = 12)

Number	Age (years)	Sex	Diagnosis	Total IgE (IU/mL)	Wheat IgE (kU$_A$/L)	Clinical Symptoms After Ingestion of Wheat (OFC Reaction)
15	1.3	F	AD, FA	151	10.9	AE
16	7.5	M	AD, AR, FA	1124	9.26	U
17	6.1	M	AD, AR, BA, FA	1422	32.9	Ana
18	1	M	AD, BA, FA	42.7	0.67	(U)
19	5	M	AD, AR, BA, FA	203	1.49	(U)
20	1	F	AD, FA	94	15.9	Ana
21	5	M	AD, AR, BA, FA	199	2.17	(U)
22	1	F	FA	116	36.6	Ana
23	3	M	AD, FA	323	11.1	U, AE
24	2	F	AD, CU, FA	219	82.6	Ana
25	2	M	AD, FA	296	97.5	Ana
26	1	M	AD, FA	289	4.07	(U, Sn)

AD = atopic dermatitis; AE = angioedema; Ana = anaphylaxis; AR = allergic rhinitis; BA = bronchial asthma; CU = chronic urticaria; FA = food allergy; (Sn) = sneezing during OFC; U = urticaria; (U) = urticaria during OFC; OFC = oral food challenge.

Glycan Detection in Gliadin

Glycans were identified using a commercially available kit (DIG Glycan Differentiation kit; Roche, Mannheim, Germany) following the manufacturer's instructions. The specific binding of lectins to carbohydrate moieties was used to identify glycan and its structure.

IgE Immunoblotting of Gliadin and Deglycosylated Gliadin

For glycan removal, gliadin was oxidized by soaking the immunoblot membrane in 20mM NaIO$_4$/50mM acetate buffer (pH 4.5) for two hours at room temperature. To evaluate gliadin-IgE binding, untreated and

NaIO$_4$-treated gliadin membranes were reacted with serum (1:10) overnight at 4°C. The membranes were incubated for one hour with alkaline phosphatase-labeled antihuman IgE antibodies (Sigma-Aldrich). The membranes were developed in nitro-blue tetrazolium/5-bromo-4-chloro-3'-indolyphosphate solution (Roche Diagnostics, Mannheim, Germany).

To confirm glycan removal, glycan detection was performed using a DIG Glycan Differentiation kit (Roche Diagnostics) following the supplier's instructions.

ELISA of Gliadin and Deglycosylated Gliadin

Gliadin was dissolved in 100mM carbonate buffer (pH 10.0). Gliadin (3 µg/mL) was coated onto 96-well microtiter plates (Costar Co., NY) overnight at 4°C and then blocked with 1% BSA in PBS for one hour at room temperature. Diluted serum (1:10) in block buffer was allowed to react for one hour at room temperature. IgE was detected by incubation with biotinylated antihuman IgE antibody (Vector Laboratories, Burlingame, CA) for one hour at room temperature, followed by incubation with streptavidin-conjugated horseradish peroxidase (R&D Systems, Minneapolis, MN) for 30 minutes. The plates were developed by addition of the TMB substrate (KPL, Inc., Gaithersburg, MD). The absorbance at 450 nm was measured with a VersaMax microplate reader (Molecular Devices, Sunnyvale, CA).

To remove glycan, gliadin adsorbed onto microtiter plates was oxidized by adding 40mM NaIO$_4$ in 50mM acetate buffer (pH 4.5).

Statistical Analysis

Data were expressed as mean ± SD. Statistical analysis comparing gliadin, gliadin ratio, and allergenicity of glycan among different groups were assessed by Student's t-test. Statistical analysis comparing IgE binding with gliadin and to deglycosylated gliadin was assessed by paired t-test. Correlation coefficients were determined using the Spearman rank correlation test. $p < 0.05$ was considered significant. T.W.S., J.Y.H., and K.E.L. contributed equally to this work.

RESULTS

Proteins, Glycans, and IgE Binding of Gliadin

Protein bands of gliadin ranging from 25 to 60 kDa were observed (Fig. 2). We observed binding between gliadin and IgE from wheat-positive pooled serum through the entire molecular weight range of gliadin. However, IgE binding was not observed with either negative pooled serum or buffer control (Fig. 2). The range of glycan in gliadin overlapped almost completely with that of gliadin itself (Fig. 2).

M: molecular weight marker, lane1: gliadin, lane 2: gliadin, lane 3: wheat-positive pooled serum, lane 4: negative pooled serum, lane 5: buffer control
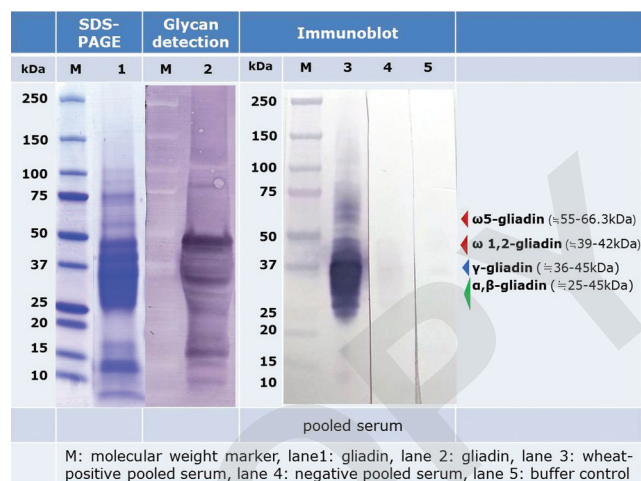
Figure 2. Protein bands of gliadin ranging from 25 to 60 kDa, separated by SDS-PAGE. The glycan component of gliadin was detected by the DIG Glycan Differentiation kit, and its molecular weight range overlapped almost completely with gliadin, from 25 to 60 kDa. IgE binding to gliadin in wheat-positive pooled serum through the entire molecular weight range of gliadin were observed by immunoblotting.

Correlation of Wheat-Specific IgE and Gliadin-Specific IgE in Wheat Allergy

The level of wheat-specific IgE, as detected by the ImmunoCAP test, was significantly correlated with the level of gliadin-specific IgE detected by ELISA in the symptomatic, never-exposed, and asymptomatic groups ($r = 0.64$, $p = 0.026$, $r = 0.75$, $p = 0.020$ and $r = 0.90$, $p = 0.137$, respectively). Although not statistically significant, the symptomatic group had a higher proportion of gliadin than that seen in the never-exposed and the asymptomatic groups (0.030 versus 0.026, $p = 0.699$ and 0.030 versus 0.025, $p = 0.812$, respectively).

Allergenicity of Gliadin

In the symptomatic, never-exposed, and asymptomatic groups, IgE reactivity to gliadin was detected in all sera and was significantly higher than in the negative pooled serum (0.98 ± 0.68 versus 0, $p = 0.001$). IgE reactivity for gliadin was not observed in the negative pooled serum.

Effect of Deglycosylation on Gliadin Allergenicity

IgE binding to gliadin in wheat-positive pooled serum was detected by immunoblotting and revealed bands ranging from 25 to 60 kDa. After glycan removal by NaIO$_4$ treatment, the intensity of the bands decreased. In the never-exposed and asymptomatic groups, IgE binding was observed in all individual serum samples with bands ranging from 10 to 100 kDa in immunoblotting. After glycan removal by NaIO$_4$ treatment, the intensity of these bands decreased in most sera. However, for some sera, the visual decrease

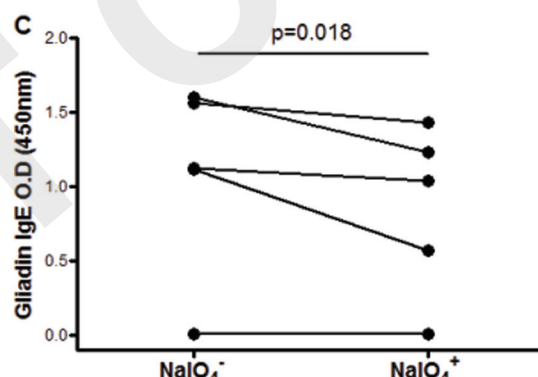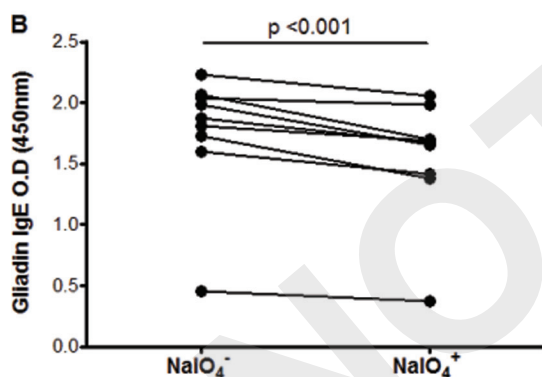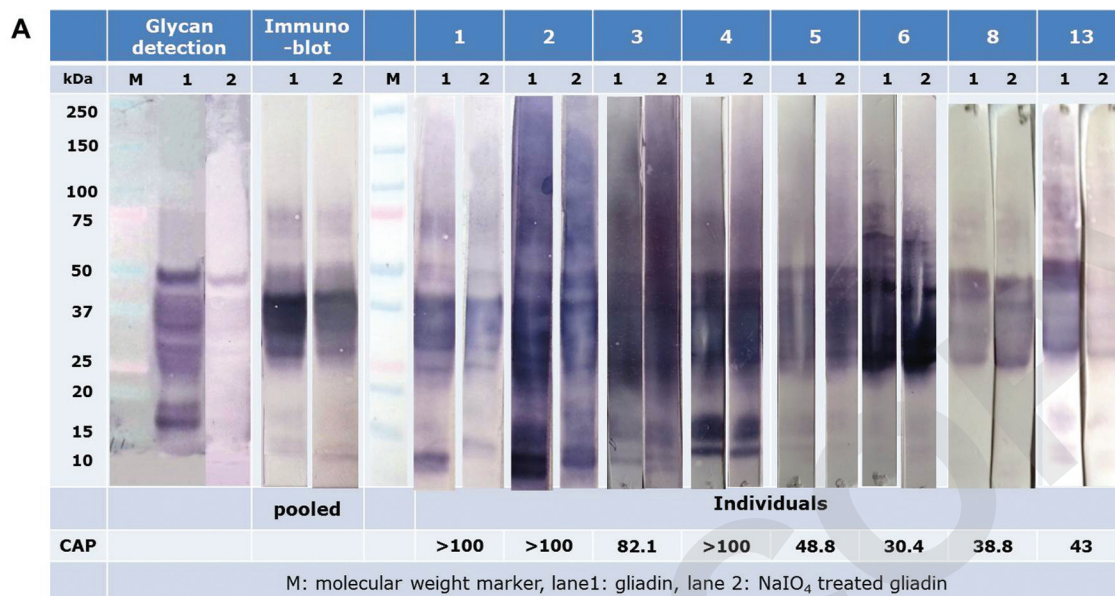|	Glycan detection			Immuno-blot			1		2		3		4		5		6		8		13	
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250																						
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CAP							>100		>100		82.1		>100		48.8		30.4		38.8		43	
			M: molecular weight marker, lane1: gliadin, lane 2: NaIO₄ treated gliadin																			

Figure 3. Comparison of IgE binding between gliadin and deglycosylated gliadin with sera from the never-exposed (number 1–9) and asymptomatic (number 10–14) groups, detected by immunoblotting and ELISA. (A) IgE binding was demonstrated in the range 10 to 100 kDa by immunoblotting. After glycan removal by NaIO₄ treatment, the band intensity decreased. (B) IgE binding to gliadin measured by ELISA showed a significant decrease after glycan removal in the never-exposed group. (C) IgE binding to gliadin measured by ELISA showed a significant decrease after glycan removal in the asymptomatic group.

was not apparent (Fig. 3 A). Therefore, we reevaluated this decrease with ELISA. In both the never-exposed and asymptomatic groups, deglycosylated gliadin was significantly less recognized by IgE antibodies compared with untreated gliadin [1.75 ± 0.52 versus 1.55 ± 0.49, $p < 0.001$ (Fig. 3 B); 1.09 ± 0.64 versus 0.86 ± 0.57, $p = 0.018$ (Fig. 3 C)].

In the symptomatic group, IgE binding was observed in all individual serum samples, with bands ranging from 25 to 60 kDa detected by immunoblotting (Fig. 4 A). After glycan removal by NaIO₄ treatment, the intensity of these bands decreased in most sera. However, for some sera, an obvious visual decrease was not observed (Fig. 4 A). Therefore, we confirmed that deglycosylated gliadin was significantly less recognized by IgE antibodies compared with gliadin, in all sera from the symptomatic group, by ELISA (0.36 ± 0.35 versus 0.24 ± 0.29, $p < 0.001$) (Fig. 4 B).

Allergenicity of the Glycan Portion in Gliadin

The binding of IgE to the glycan portion of gliadin was more pronounced in the symptomatic group than in the never-exposed group (0.34 ± 0.19 versus 0.12 ± 0.06, $p = 0.004$) (Fig. 5). Although not statistically significant, the binding of IgE to the glycan portion of gliadin appeared to be higher in the symptomatic group than in the asymptomatic group (0.34 ± 0.19 versus 0.24 ± 0.17, $p = 0.341$) (Fig. 5).

DISCUSSION

This is the first study to investigate the allergenicity of glycan, which is present in gliadin as a carbohydrate epitope, in children with wheat allergy.

Previously, various levels of IgE reactivities to gliadins were detected in children with food allergies to wheat. Mittag *et al.* reported that seven of eight chil-
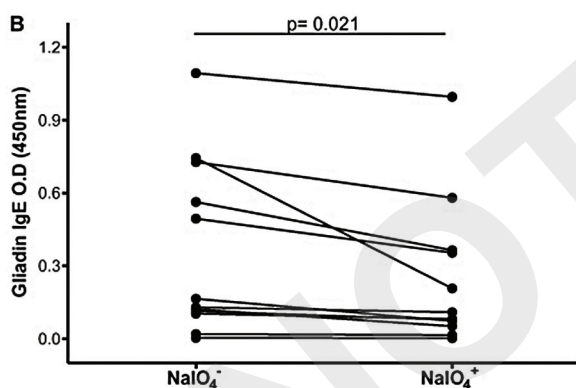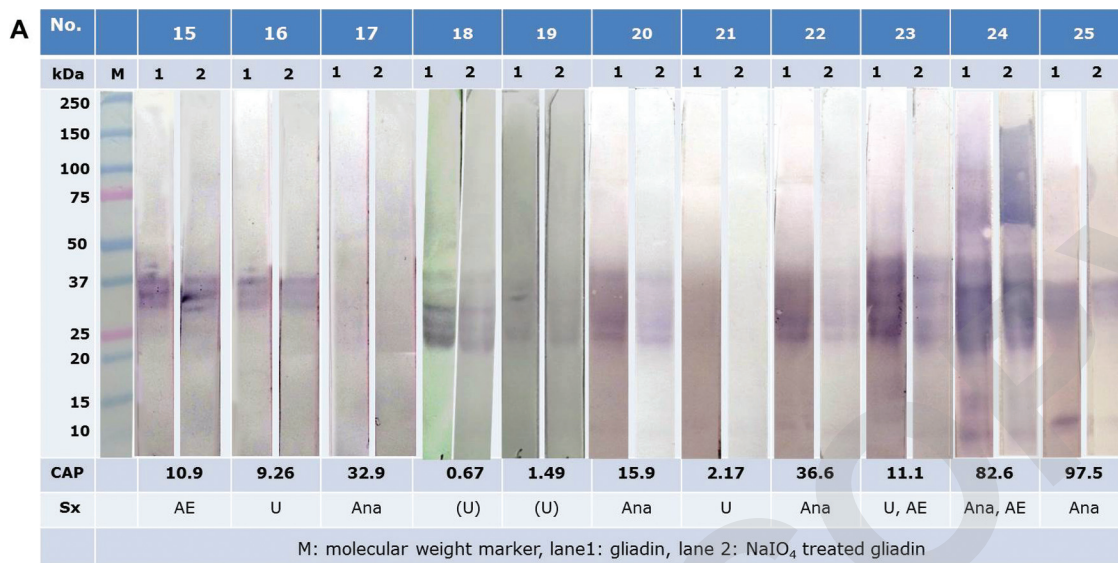
Figure 4. *Comparison of IgE binding between gliadin and deglycosylated gliadin in sera from symptomatic group, detected by immunoblotting and ELISA. (A) IgE binding was demonstrated in the 25 to 60 kDa by immunoblotting. After glycan removal by NaIO₄ treatment, the band intensity decreased. (B) IgE binding to gliadin as measured by ELISA was significantly decreased after glycan removal. AE, angioedema; Ana, anaphylaxis; U, urticaria; (U), urticaria during OFC.*

dren with food allergy to wheat exhibited gliadin-specific IgE in immunoblotting experiments,[16] and Battais *et al.* revealed that of 27 patients (14 adults/14 children) with wheat allergies, the percentage of patients with specific IgE to α-gliadin was 7%, to β-gliadin was 20%, to γ-gliadin was 7%, and to fast ω-gliadin was 20% in immunoblotting studies.[15] In this study, all patients with wheat allergy (*i.e.,* the symptomatic group) showed IgE reactivity to whole gliadin, although two patients showed very weak activity. Our results demonstrate the importance of whole gliadin as an allergen for children with wheat allergy, consistent with previously published data. The differences in percentages between our study and previous studies could be caused by the levels of wheat-specific IgE in the study population, because wheat-specific IgE levels correlated with gliadin-specific IgE levels in our study. IgE reactivity to gliadin might reflect wheat-specific IgE levels and this could be anticipated, because gliadin is a fraction of wheat proteins.

Some wheat proteins belonging to both the gliadin and glutenin families showed reactivity to antibodies against xylose containing N-glycans. Therefore, these proteins are thought to be glycosylated.[25] However, to our knowledge, there has been no previous study investigating the range of glycosylation of gliadins. We tried to determine the range of gliadins that were glycosylated and observed that the detected glycan overlapped with almost the entire range of gliadins. Therefore, we concluded that the entire range of gliadin contains glycan.

With respect to carbohydrate allergenicity in wheat allergy, there have been a few studies examining IgE binding to well-recognized CCDs, such as horseradish peroxidase and the N-glycan of bromelain (MUXF), in Baker's asthma.[4,26] In this study, deglycosylated gliadin was significantly less recognized by IgE antibodies compared with intact gliadin, and these results suggest that glycan may be the carbohydrate epitope of gliadin. The decreased reactivity of deglycosylated gliadin
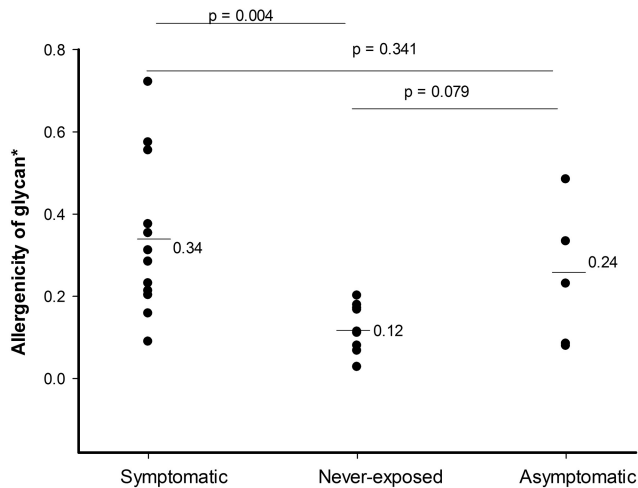
Figure 5. *Comparison of the allergenicity of the glycan portion of gliadin among the symptomatic, never-exposed, and asymptomatic groups. IgE binding to the glycan portion was greater in symptom than in the never-exposed and asymptomatic groups. *, Allergenicity of glycan = (IgE binding to gliadin − IgE binding to deglycosylated gliadin)/IgE binding to gliadin.*

compared with gliadin could be caused by other un-proven structural changes of gliadin. Similar to the current study, previous studies that evaluated the significance of the carbohydrate epitope commonly used $NaIO_4$ oxidation for deglycosylation.[27,28]

In this study, IgE binding to the glycan portion of gliadin was greater in the symptomatic group than in the never-exposed and asymptomatic groups. These results also support the significance of the glycan portion of gliadin. This study suggests a possible clinical significance for the glycan of gliadin, a previously un-evaluated carbohydrate antigen.

Seven N-linked glycosylation sites have been identified in wheat protein.[29] It is unclear whether the glycan in gliadin is one of well-recognized CCDs. However, based on our results, the immunologic significance of glycan in gliadin appears to be different from that of well-recognized CCDs, because IgE antibodies to plant-derived CCDs usually exhibit only minor clinical significance and lack specificity.[19]

In conclusion, whole gliadin could be considered an important allergen for children with wheat allergy, and the entire range of gliadins are glycosylated. Deglyco-sylation of gliadin reduced the allergenicity of gliadin. The N-glycan moiety of gliadin might exhibit allergenicity and act as a carbohydrate epitope in wheat allergy in children.

REFERENCES

1. Imai T. The national survey of immediate type of food allergy [in Japanese]. Arerugi 53:689–695, 2004.
2. Robison RG, and Pongracic JA. Chapter 23: food allergy. Allergy Asthma Proc 33:S77–S79, 2012.
3. Salcedo G, Quirce S, and Diaz-Perales A. Wheat allergens associated with Baker's asthma. J Investig Allergol Clin Immunol 21:81–92; quiz 94, 2011.
4. Sander I, Rozynek P, Rihs HP, et al. Multiple wheat flour allergens and cross-reactive carbohydrate determinants bind IgE in baker's asthma. Allergy 66:1208–1215, 2011.
5. Shewry PR, Tatham AS, and Halford NG. The prolamins of the Triticeae. In Seed Proteins. Shewry PR, and Casey R (Eds). Dordrecht: Kluwer Academic Publishers, 35–78, 1999.
6. Bittner C, Grassau B, Frenzel K, and Baur X. Identification of wheat gliadins as an allergen family related to baker's asthma. J Allergy Clin Immunol 121:744–749, 2008.
7. Sandiford CP, Tatham AS, Fido R, et al. Identification of the major water/salt insoluble wheat proteins involved in cereal hypersensitivity. Clin Exp Allergy 27:1120–1129, 1997.
8. Morita E, Kameyoshi Y, Mihara S, et al. γ-Gliadin: a presumptive allergen causing wheat-dependent exercise-induced anaphylaxis. Br J Dermatol 145:182–184, 2001.
9. Lehto M, Palosuo K, Varjonen E, et al. Humoral and cellular responses to gliadin in wheat-dependent, exercise-induced anaphylaxis. Clin Exp Allergy 33:90–95, 2003.
10. Morita E, Matsuo H, Mihara S, et al. Fast omega-gliadin is a major allergen in wheat-dependent exercise-induced anaphylaxis. J Dermatol Sci 33:99–104, 2003.
11. Park HB, Choi BS, Kim MN, et al. A case of gluten allergy in a 4-year-old boy with recurrent urticaria. Pediatr Allergy Respir Dis (Korea) 20:292–296, 2010.
12. Ito K, Futamura M, Borres MP, et al. IgE antibodies to omega-5 gliadin associate with immediate symptoms on oral wheat challenge in Japanese children. Allergy 63:1536–1542, 2008.
13. Palosuo K, Varjonen E, Kekki OM, et al. Wheat omega-5 gliadin is a major allergen in children with immediate allergy to ingested wheat. J Allergy Clin Immunol 108:634–638, 2001.
14. Ebisawa M, Shibata R, Sato S, et al. Clinical utility of IgE antibodies to ω-5 gliadin in the diagnosis of wheat allergy: a pediatric multicenter challenge study. Int Arch Allergy Immunol 158:71–76, 2012.
15. Battais F, Pineau F, Popineau Y, et al. Food allergy to wheat: identification of immunogloglin E and immunoglobulin G-binding proteins with sequential extracts and purified proteins from wheat flour. Clin Exp Allergy 33:962–970, 2003.
16. Mittag D, Niggemann B, Sander I, et al. Immunoglobulin E-reactivity of wheat-allergic subjects (baker's asthma, food allergy, wheat-dependent, exercise-induced anaphylaxis) to wheat protein fractions with different solubility and digestibility. Mol Nutr Food Res 48:380–389, 2004.
17. Varki A, Cummings RD, Esko JD, et al. Essentials of Glycobiology. 2nd ed. New York, NY: Cold Spring Harbor Laboratory Press, 1–22, 2009.
18. Commins SP, and Platts-Mills TAE. Allergenicity of carbohydrates and their role in anaphylactic events. Curr Allergy Asthma Resp 10:29–33, 2010.
19. Aalberse RC, Koshte V, and Clemens JG. Immunoglobulin E antibodies that crossreact with vegetable foods, pollen, and Hymenoptera venom. J Allergy Clin Immunol 68:356–364, 1981.
20. Altmann F. The role of protein glycosylation in allergy. Int Arch Allergy Immunol 142:99–115, 2007.
21. Chung CH, Mirakhur B, Chan E, et al. Cetuximab-induced anaphylaxis and IgE specific for galactose-α-1,3-galactose. N Engl J Med 358:1109–1117, 2008.
22. Commins SP, Satinover SM, Hosen J, et al. Delayed anaphylaxis, angioedema, or urticaria after consumption of red meat in

patients with IgE antibodies specific for galactose-α-1,3-galactose. J Allergy Clin Immunol 123:426–433, 2009.

23. Sampson HA. Food allergy–accurately identifying clinical reactivity. Allergy 60:19–24, 2005.

24. Song TW, Kim KW, Kim WK, et al. Atopic Dermatitis Study Group, The Korean Acedemy of Pediatric Allergy and Respiratory Disease. Guidelines for the oral food challenges in children. Pediatr Allergy Respir Dis (Korea) 22:3–20, 2012.

25. Snégaroff J, Bouchez I, Smaali Mel A, et al. Barley γ3-hordein: glycosylation at an atypical site, disulfide bridge analysis, and reactivity with IgE from patients allergic to wheat. Biochim Biophys Acta 1834:395–403, 2013.

26. Tanabe S, Tesaki S, Watanabe M, and Yanagihara Y. [Cross-reactivity between bromelain and soluble fraction from wheat flour]. Arerugi 46:1170–1173, 1997.

27. Fötisch K, Fäh J, Wüthrich B, et al. IgE antibodies specific for carbohydrates in a patient allergic to gum arabic (*Acacia senegal*). Allergy 53:1043–1051, 1998.

28. Yagami T, Osuna H, Kouno M, et al. Significance of carbohydrate epitopes in a latex allergen with β-1,3-glucanase activity. Int Arch Allergy Immunol 129:27–37, 2002.

29. Dionisio G, Brinch-Pedersen H, Welinder KG, et al. Different site-specific N-glycan types in wheat (*Triticum aestivum* L.) PAP phytase. Phytochem 72:1173–1179, 2011. □

Erratum

In the article *Serum interleukin 17, interleukin 23, and interleukin 10 values in children with atopic eczema/dermatitis syndrome (AEDS): Association with clinical severity and phenotype,* Allergy Asthma Proc 36, 74–81, 2015; doi: 10.2500/aap.2015.36.3808, in Table 2 there were some incorrect values within the columns. The revised table is below.

The authors regret the error.

Table 2. **Total IgE, IL-17, IL-23, and IL-10 in aAEDS, naAEDS subjects, and heathy controls**

	N	SCORAD	Total IgE UI/mL	IL-17 pg/mL	IL-23 pg/mL	IL-10 pg/mL
aAEDS	104		360.7 ± 25.34	72.49 ± 10.00	235.84 ± 65.07	3.28 ± 0.89
Mild	32	20.31 ± 2.2	333.24 ± 23.59	59.30 ± 3.219	180.57 ± 12.25	3.88 ± 0.48
Moderate	37	39.76 ± 9.03	366.02 ± 7.67	74.08 ± 2.54	233.67 ± 13.20	3.678 ± 0.50
Severe	35	56.67 ± 4.52	385.25 ± 6.42	82.87 ± 3.07	288.96 ± 9.58	2.19 ± 0.25
naAEDS	77		69.80 ± 12.29	53.94 ± 0.95	188.02 ± 11.75	3.15 ± 0.22
Mild	25	21.33 ± 2.1	68.37 ± 11.46	50.01 ± 0.86	148.66 ± 8.15	3.91 ± 0.23
Moderate	29	37.21 ± 8.64	67.75 ± 15.45	53.86 ± 0.75	190.12 ± 14.8	3.63 ± 0.20
Severe	23	54.31 ± 2.11	73.3 ± 9.7	57.97 ± 1.25	225.3 ± 12.3	1.93 ± 0.24
Control group	93	—	73.4 ± 20.3	29.3 ± 12.1	111.3 ± 13.4	4.95 ± 0.5

AEDS = atopic eczema/dermatitis syndrome; aAEDS = atopic AEDS; IL = interleukin; naAEDS = nonatopic AEDS; SCORAD = Score Atopic Dermatitis.