

ORIGINAL ARTICLE

WILEY

Cross-reactivity of each fraction among cereals in children with wheat allergy

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Funding information

The work was supported by funding from the Research on Food Labeling Related to Food Allergy in 2016, Consumer affairs agency, Government of Japan.

Editor: Alexandra Santos

Abstract

Background: Cross-reactivity between wheat and other cereals is a crucial issue in the management of wheat allergy. Few studies have reported in vitro cross-reactivity in immediate-type wheat allergy. The aim of this study aimed to examine cross-reactivity of the three fractions (albumin/globulin, gliadin, and glutenin fractions) among cereals in children with wheat allergy.

Methods: Sera from 128 children with immediate-type wheat allergy were collected. Specific immunoglobulin E (sIgE) levels against each fraction of wheat, barley, and rye were measured by enzyme-linked immunosorbent assay (ELISA). Cross-reactivities of each fraction among wheat, barley, and rye were examined via inhibition ELISA.

Results: All subjects were sensitized to all fractions of wheat, barley, and rye. The wheat sIgE levels were significantly higher than those of barley and rye in all the fractions ($p \leq .001$) and were significantly correlated with sIgE levels in each fraction ($r = .887-.969$, $p < .001$). Inhibition ELISA revealed that wheat inhibited the IgE binding to most of the solid phases at lower protein levels compared with barley and rye in all fractions.

Conclusions: In children with immediate-type wheat allergy, sensitization to all the three fractions of wheat was observed. In addition, they showed sensitization to barley and rye caused by in vitro cross-reactivity with wheat in each fraction. When managing children with wheat allergy, sensitization to barley and rye caused by the cross-reactivities should be considered.

KEYWORDS

albumin/globulin, barley, cross-reactivity, gliadin, glutenin, immediate-type wheat allergy, rye

1 | INTRODUCTION

Wheat allergy is a food allergy with different clinical types, including immediate-type, wheat-dependent exercise-induced anaphylaxis (WDEIA), baker's asthma, and celiac disease.^{1,2} In Japan, the immediate-type wheat allergy is one of the most common types of food allergy among children.³ Cereals, including wheat, are used as food material worldwide.⁴ Cross-reactivity among cereals is an

important issue in the management of wheat allergy.^{5,6} Examination of in vitro cross-reactivity among cereals might be helpful in considering clinical cross-reactivity.

Wheat proteins are classified into three fractions: (a) the water/salt-soluble fraction: the albumin/globulin (alb/glob) fraction, (b) the alcohol-soluble fraction: the gliadin fraction, and (c) the alcohol-insoluble fraction: the glutenin fraction.⁷ In baker's asthma, α -amylase inhibitor and peroxidase, belonging to the alb/glob fraction,

have been considered major allergens.^{8,9} A gliadin protein, ω -5 gliadin, has been identified as a major allergen in WDEIA and among children with immediate-type wheat allergy.^{10–13} Furthermore, glutenin is an allergen in patients with immediate-type wheat allergy and WDEIA.^{14,15} These reports indicate that various wheat proteins in each fraction can act as causative allergens in wheat allergy.

Wheat (*Triticum aestivum*) belongs to the Triticeae tribe in the Poaceae family. Barley (*Hordeum vulgare*) and rye (*Secale cereale*) are also members of the same tribe. Some in vitro studies have evaluated cross-reactivity among wheat, barley, and rye in adult patients. In a study on baker's asthma, cross-reactivity in the alb/glob fraction among these cereals was confirmed in a competitive radioallergosorbent inhibition assay.¹⁶ Another study reported that most patients with WDEIA were sensitive to r-3 hordein (barley), r-70, and r-35 secalin (rye), which cross-react with ω -5 gliadin according to enzyme-linked immunosorbent assay (ELISA).¹⁷

However, limited information is available regarding the cross-reactivity of each fraction in immediate-type wheat allergy. Therefore, we performed an in vitro assay to clarify the cross-reactivity of each fraction among wheat, barley, and rye in children with immediate-type wheat allergy. In addition, the allergenicity of cereals and the relationship between in vitro and clinical cross-reactivity are discussed based on the results of inhibition ELISA.

2 | METHODS

2.1 | Patient sera

Sera were obtained from 128 children (median age, 6.3 years) with sensitization to wheat who showed positive results in the wheat oral food challenge or had a clinical history of immediate allergic reaction after consuming wheat. Clinical characteristics of study subjects are shown in Table S1. We excluded sera from children with WDEIA and baker's asthma in this study. Cereal allergen-specific immunoglobulin E (sIgE) levels were measured by using the ImmunoCAP system (Thermo Fisher Scientific/Phadia). Sensitization to cereals was defined as an IgE level ≥ 0.10 kU_A/L. The median time between the last allergic reaction to wheat and measurement of sIgE levels was 7 months (IQR, 3–14 months). Serum samples were stored at -30°C for immunological analysis.

2.2 | Cereal protein extraction

Wheat, barley, and rye flour were purchased from different manufacturers. We confirmed with the manufacturers that there was little possibility of other cereals being contaminated in the manufacturing process. The protocol for protein extraction for each fraction was in accordance with that reported by Sandiford et al.¹⁸

The flours were defatted with ether and then air-dried. The alb/glob fraction was extracted in 0.125 M ammonium bicarbonate (1:10 w/v) for 48 h at 4°C and centrifuged at 15,000 g for 20 min.

Key Message

Wheat is one of the most important causative food allergens in children and adults. Wheat and other cereals are used as food materials worldwide. Therefore, when managing immediate-type wheat allergy, sensitization to barley and rye caused by the cross-reactivity to wheat in all fractions should be considered.

The supernatant (alb/glob fraction) was dialyzed against 0.005 M ammonium bicarbonate for 24 h and freeze-dried. The pellet was washed with distilled water for 30 min at 4°C and centrifuged at 5000 g for 10 min. Then, the pellet was extracted in 70% cold ethanol for 1 h at 4°C and centrifuged at 10,000 g for 15 min. The supernatant (the gliadin fraction) was dialyzed against 1% acetic acid for 24 h and freeze-dried. The pellet was washed with distilled water for 30 min at 4°C and centrifuged at 5000 g for 10 min. Then, the pellet was extracted in 50% propanol, 1% acetic acid, and 2% 2-mercaptoethanol for 1 h at 25°C and centrifuged at 10,000 g for 10 min. The supernatant (the glutenin fraction) was dialyzed in distilled water for 6 h, then dialyzed against 1% acetic acid for 24 h, and freeze-dried.

Protein concentrations in the extracted fractions (shown in Table S2) were measured using Bradford's method (DC protein assay kit, Bio-Rad Laboratories).¹⁹

2.3 | Fluorescent ELISA

Fluorescent ELISA was performed to measure sIgE levels for each cereal fraction as previously described.²⁰ Each fraction dissolved in 0.1 mol/L carbonate-bicarbonate buffer (pH 9.6) was coated for 4 h at 37°C in a polystyrene microplate (C96 WHITE MAXISORP FLUORONUNC CERT; Thermo Fisher Scientific) at a concentration of 2 μg dry weight/well, blocked with 1% bovine serum albumin in PBS for 1 h at 37°C , and incubated for 4 h at 25°C with diluted sera from the patients. Furthermore, β -galactosidase-conjugated anti-human IgE (CAP System; Thermo Fisher Scientific) was added. After incubation for 18 h at 25°C , 4-methylumbelliferyl- β -D-galactopyranoside was added to the wells and incubated for 1 h at 37°C . After the enzyme reaction was terminated with glycine-NaOH, the fluorescence intensity was read using a microplate fluorescence reader (TECAN GmbH; Salzburg, Austria) at an excitation and an emission wavelength of 360 and 465 nm, respectively.

2.4 | Inhibition ELISA

Inhibition ELISA was performed to assess in vitro cross-reactivity as previously described.²¹ First, six subjects were randomly selected, and their sera were mixed in equal proportions. Pooled sera were preincubated with each fraction of wheat, barley, and

rye (2.56 ng–1 mg dry weight/ml) as inhibitors for 1 h at 37°C. Thereafter, preincubated sera were added to microplates coated with each fraction of wheat, barley, and rye. Subsequent experiments were performed by fluorescent ELISA as described above. Inhibition analyses were performed for each fraction. IgE reactivity in the inhibition analyses was determined as a rate of inhibition, and the results were presented as inhibition curves with the protein amount of inhibitor as the horizontal axis and the rate of inhibition as the vertical axis.

2.5 | Statistical analysis

Data were analyzed using GraphPad Prism 6 (GraphPad Software Inc.). Medians and IQRs were determined, and Kruskal–Wallis tests were performed for continuous variables. Spearman's correlation coefficient was used to determine the relationships among continuous variables. A $p < .05$ was considered statistically significant.

2.6 | Ethical consideration

The study was performed in accordance with the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participants or their guardians. The Ethics Committee of the National Hospital Organization Sagami Hospital approved this study (approval no. 160106).

3 | RESULTS

3.1 | Sensitization against each fraction

For sIgE levels measured using fluorescent ELISA, all subjects were sensitized to the three fractions of wheat. They reacted to each fraction of barley and rye, although some cases had low sIgE levels against the alb/glob fraction. The wheat sIgE levels of all fractions were significantly higher than those of barley and rye ($p \leq .001$, Figure 1). No significant differences in sIgE levels were observed between barley

and rye. Strong correlations among the sIgE levels against these cereals were observed in all the fractions ($r = .887-.969$, $p < .001$, Figure 2).

sIgE levels measured using the ImmunoCAP system, revealed similar results as those measured using ELISA. They also showed sensitization to barley and rye. The median (IQR) sIgE levels against wheat, barley, and rye were 32.8 kU_A/L (12.9–159 kU_A/L), 11.1 kU_A/L (3.10–38.3 kU_A/L), and 14.0 kU_A/L (4.50–61.1 kU_A/L), respectively. Wheat sIgE levels were significantly higher than those of barley and rye ($p < .001$, Figure S1A), and the sIgE levels of these cereals showed strong correlations ($r = .949-.960$, $p < .001$, Figure S1B).

In this study, cross-reactivity of wheat to barley and rye was 100% from the viewpoint of sensitization.

3.2 | Inhibition ELISA in each fraction

Figure 3 shows the results of the inhibition assay for each fraction using pooled sera.

In the alb/glob fraction (Figure 3A–C), all cereals inhibited IgE binding by over 50% to all solid phases in a dose-dependent manner. Furthermore, wheat inhibited IgE binding at the lower protein levels for all solid phases.

In the gliadin fraction (Figure 3D–F) and glutenin fraction (Figure 3G–I), wheat inhibited IgE binding to barley and rye solid phases, as in the alb/glob fraction. In the wheat solid phase, barley and rye did not inhibit IgE binding to wheat (Figure 3D,G). In the barley gliadin and glutenin solid phases (Figure 3E,H), the protein levels required for 50% inhibition were higher in barley than in wheat and rye.

3.3 | Relationship between clinical and in vitro cross-reactivity

Of the 128 patients, 18 (14%) were allergic to barley (barley-allergic group) and 35 (27%) were able to consume barley (barley-tolerant group). The remaining 75 (59%) had never consumed barley. No one had ever consumed rye.

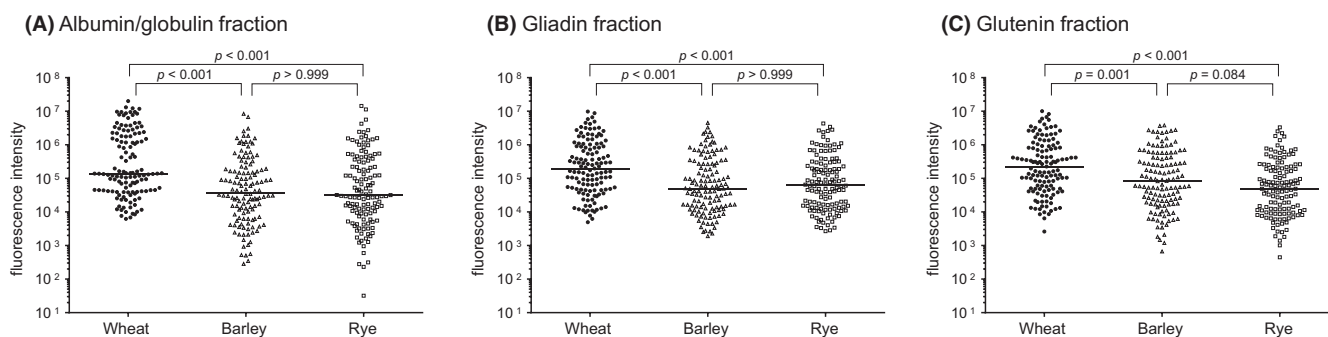


FIGURE 1 Distribution of wheat, barley, and rye sIgE levels in the three fractions. Cereal sIgE levels in albumin/globulin (A), gliadin (B), and glutenin (C) fraction were measured by fluorescent ELISA. The wheat sIgE levels of all fractions were significantly higher than those of barley and rye. ELISA, enzyme-linked immunosorbent assay; sIgE, specific immunoglobulin E

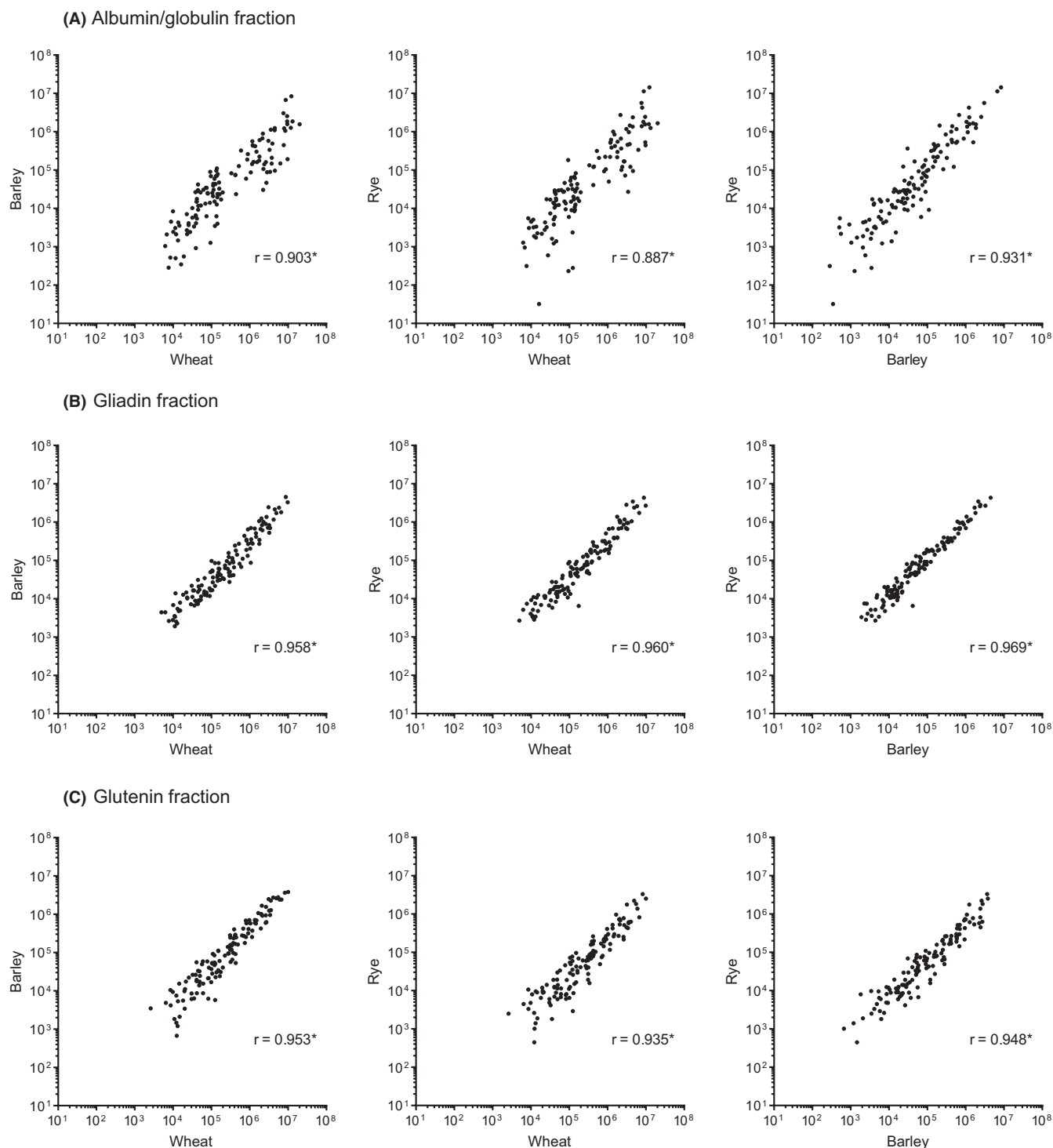


FIGURE 2 Correlations among wheat, barley, and rye sIgE levels in the three fractions. Correlations among cereal sIgE levels in albumin/globulin (A), gliadin (B), and glutenin (C) fraction are shown. There were significant correlations among cereals. $*p < .001$; sIgE, specific immunoglobulin E

The barley sIgE levels were significantly higher in all the three fractions in the barley-allergic group ($p < .001$, Figure S2). The correlation coefficients between wheat sIgE and barley sIgE in the three fractions were not significantly different between the two groups (data not shown).

Inhibition ELISA of barley using wheat as an inhibitor showed differences in the inhibition pattern between barley-allergic and -tolerant groups in all the three fractions. The differences were more pronounced in the gliadin and glutenin fractions than in the alb/glob fraction (Figure 4).

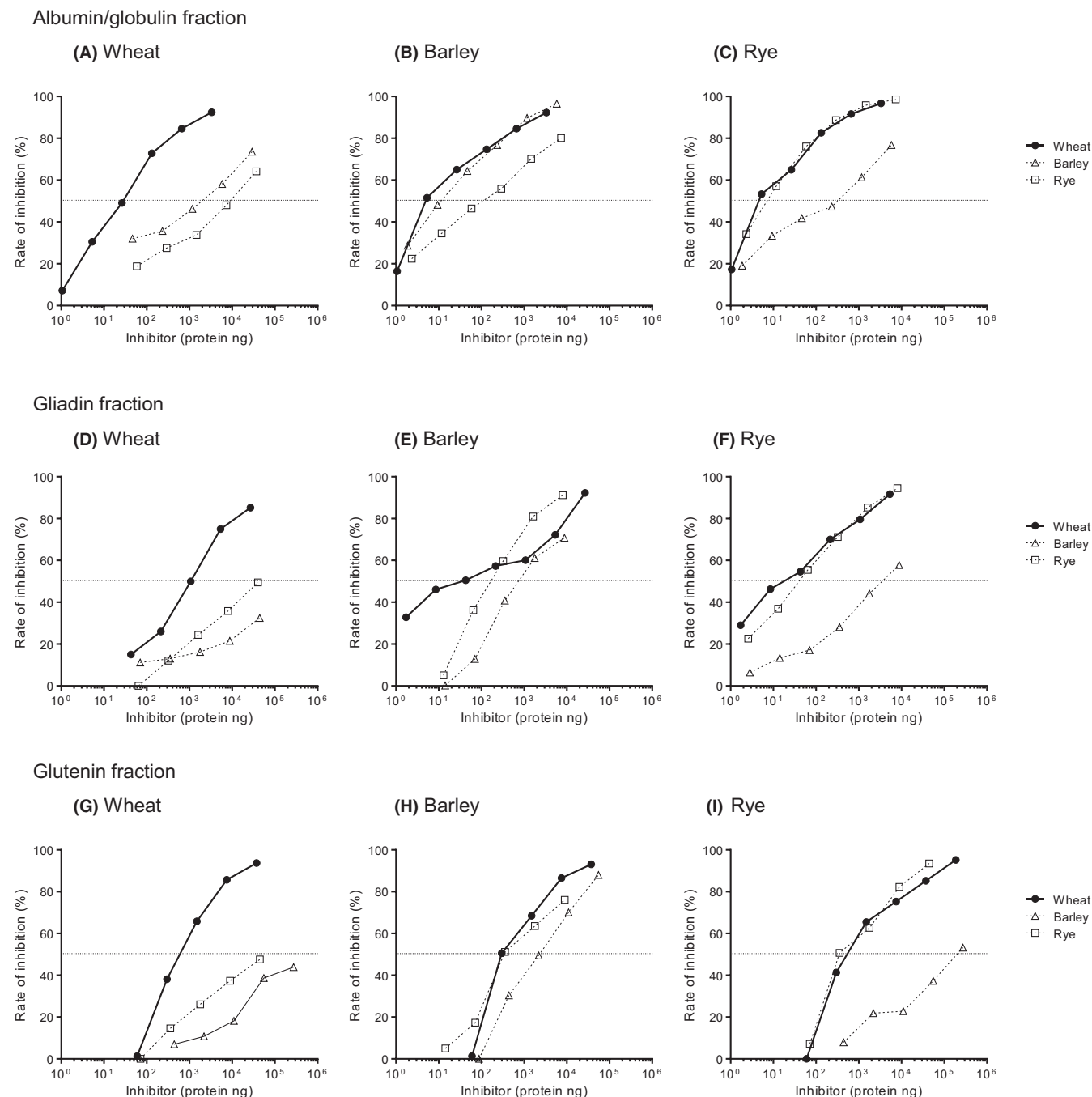


FIGURE 3 Inhibition ELISA curve of each fraction among wheat, barley, and rye. The horizontal axis indicates the protein amount of inhibitor; the vertical axis indicates the rate of inhibition. The dotted straight line shows the 50% inhibition rate. Solid phases were albumin/globulin fraction (A–C), gliadin fraction (D–F), and glutenin fraction (G–I). Wheat (closed circle with solid line), barley (open triangle with dotted line), and rye (open square with dotted line) were used as inhibitors for each solid phase; wheat (A,D,G), barley (B,E,H), and rye (C,F,I). ELISA, enzyme-linked immunosorbent assay

4 | DISCUSSION

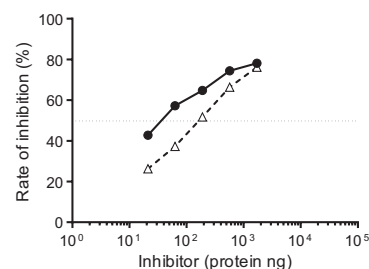
The results of this study revealed that children with immediate-type wheat allergy had sensitization to all the three fractions of wheat, and cross-reactivity to barley, and rye in all the three fractions. Moreover, the allergenicity of wheat appeared to be higher than that of barley and rye, suggesting that wheat is a primary source of

sensitization, and sensitization to barley and rye was caused by the cross-reactivity. To the best of our knowledge, this is the first study to clarify the in vitro cross-reactivity of each fraction among cereals in immediate-type wheat allergy.

All subjects were sensitized to barley and rye. The sIgE levels against wheat were significantly higher than those of barley and rye. Furthermore, strong correlations among the sIgE levels were

Albumin/globulin fraction

(A) Inhibitor: wheat



(B) Inhibitor: barley

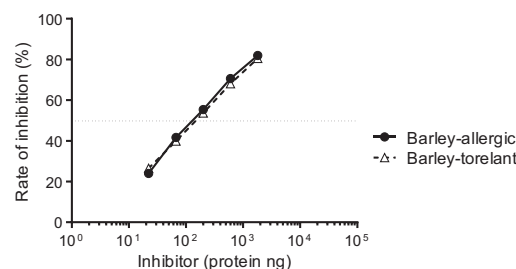
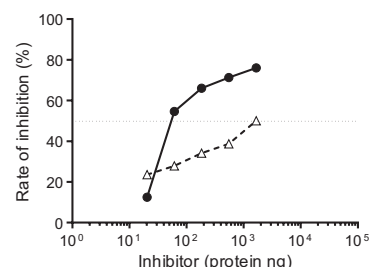


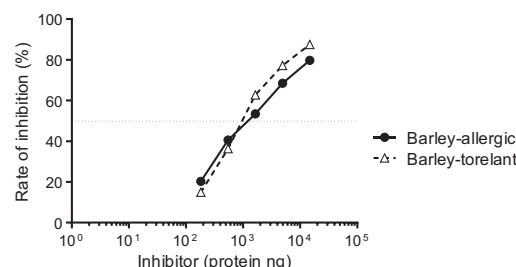
FIGURE 4 Inhibition ELISA curve of each fraction among barley-allergic and -tolerant groups. The horizontal axis indicates the protein amount of inhibitor; the vertical axis indicates the rate of inhibition. The dotted straight line shows the 50% inhibition rate. Solid phases were barley of each fraction. Wheat (A,C,E) and barley (B,D,F) were used as inhibitors for each fraction. ELISA, enzyme-linked immunosorbent assay

Gliadin fraction

(C) Inhibitor: wheat

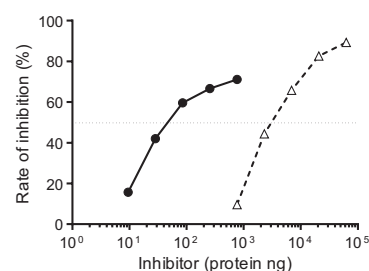


(D) Inhibitor: barley

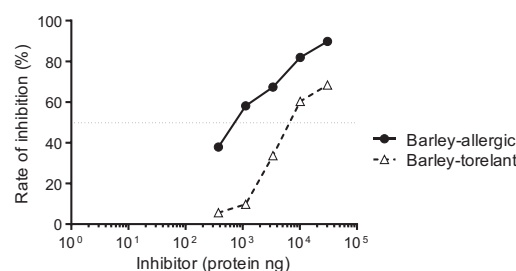


Glutenin fraction

(E) Inhibitor: wheat



(F) Inhibitor: barley



observed among wheat, barley, and rye. Sandiford et al. measured the sIgE levels of cereal alb/glob fractions in 31 patients with baker's asthma through a competitive radioallergosorbent inhibition assay and reported that 94% and 97% of the patients were sensitized to barley and rye, respectively. Furthermore, the sIgE levels against these cereals were highly correlated with each other.¹⁶ In a previous study on children with wheat allergy ($n = 18$), sensitization to barley was observed in 89% of the subjects, and the correlation of sIgE levels between wheat and barley, as measured using the ELISA method, were relatively high.²² We obtained similar results among wheat, barley, and rye, with strong correlations in each fraction and in a larger population.

In vitro cross-reactivity among cereals was previously evaluated in the alb/glob fraction among patients with baker's asthma^{16,23} and in the gliadin fraction among adult patients with WDEIA.^{17,24} In the present study, we showed that wheat has cross-reactivity with barley and rye in all the fractions among children with immediate-type wheat allergy, suggesting that IgE-binding epitopes in barley and rye might be shared with those in wheat. Moreover, the results of

inhibition ELISA showed that wheat inhibited IgE binding to barley and rye solid phases with lower amounts of protein than barley and rye in all the fractions. Similar results were observed in the gliadin fraction from patients with WDEIA.¹⁷ The results of the present study suggest that wheat has the strongest allergenicity among wheat, barley, and rye in children with immediate-type wheat allergy. Wheat sIgE levels were significantly higher than those of barley and rye, thereby supporting these results. Thus, we considered that wheat is a primary source of sensitization; barley and rye result in secondary sensitization owing to cross-reactivity to wheat. Regarding the relationship between barley and rye, barley proteins exhibited 50% inhibition at higher amounts than rye protein in the barley gliadin and barley glutenin fractions, indicating that the allergenicity of rye might be higher than that of barley in the gliadin and glutenin fractions in our study population.

All children in our study showed sensitization to other cereals, indicating only in vitro cross-reactivity, not clinical cross-reactivity. Srisuwatchari et al. described that 6 of 10 wheat-allergic children had allergy to barley based on large skin prick test diameters or

previous history and showed in vitro cross-reactivity to barley in the gliadin and glutenin fractions.²⁵ Another study showed that children with wheat allergies had 56% clinical cross-reactivity to barley in an oral food challenge.²² For other cereals, Jones et al. reported that 20% of the patients with cereal allergy reacted to two or more cereals.²⁶ These results suggest that some patients with wheat allergy may show allergic symptoms to other cereals, whereas others may be able to consume the cereals without allergic symptoms. In the present study, only less than half of the patients had ever consumed barley grain, and approximately 1/3 of them were allergic to barley. The barley-allergic group in our study had significantly higher barley sIgE in all three fractions. Furthermore, inhibition ELISA showed that all three fractions of barley were inhibited by wheat inhibitors at lower concentrations in the barley-allergic group than in the barley-tolerant group. The results were particularly marked in the gliadin and glutenin fractions. This suggests that clinical cross-reactivity to barley might correlate with high levels of barley sIgE as well as in vitro cross-reactivity in the gliadin and glutenin fractions between wheat and barley.

This study has some limitations. First, we did not evaluate cross-reactivity to cereals other than barley and rye (e.g., oat, corn, and rice, which belong to the Poaceae family). Further studies are needed to clarify the cross-reactivity between wheat and other cereals. Second, only children who developed immediate-type wheat allergy were eligible for this study. Varying results might be obtained for patients who are only allergic to barley or rye and are not allergic to wheat. However, no such patients were present in our hospital during the study period, and to our knowledge, no studies to date have focused on these patient populations. Finally, our study mainly showed in vitro cross-reactivity among cereals. Since barley and rye are not commonly consumed in Japan, we could not accurately examine clinical cross-reactivity among cereals. For managing wheat allergies, it may also be important to investigate whether patients are at a risk of other cereal allergies. Therefore, further studies are required to evaluate the clinical cross-reactivity among these cereals.

In conclusion, wheat is the primary source of sensitization, and sensitizations to all the three fractions were observed in children with immediate-type wheat allergy. We also found in vitro cross-reactivity of all fractions among wheat, barley, and rye in all children in this study. In the management of children with wheat allergy, it is important to consider sensitizations to other cereals caused by in vitro cross-reactivity. Further studies are expected to assess the clinical cross-reactivity among cereals.

AUTHOR CONTRIBUTIONS

Mari Takei: Conceptualization (lead); data curation (lead); formal analysis (lead); funding acquisition (supporting); investigation (lead); methodology (lead); project administration (lead); resources (equal); software (equal); supervision (equal); validation (equal); visualization (equal); writing – original draft (lead); writing – review and editing (equal). **Akemi Saito:** Conceptualization (equal); data curation (equal); formal analysis (equal); funding acquisition (supporting);

investigation (equal); methodology (lead); project administration (supporting); resources (equal); software (supporting); supervision (equal); validation (equal); visualization (equal); writing – original draft (equal); writing – review and editing (supporting). **Noriyuki Yanagida:** Conceptualization (supporting); data curation (supporting); formal analysis (supporting); funding acquisition (supporting); investigation (supporting); methodology (supporting); project administration (equal); resources (equal); software (supporting); supervision (lead); validation (lead); visualization (equal); writing – original draft (equal); writing – review and editing (equal). **Sakura Sato:** Conceptualization (supporting); data curation (supporting); formal analysis (supporting); funding acquisition (equal); investigation (supporting); methodology (supporting); project administration (equal); resources (equal); software (supporting); supervision (lead); validation (lead); visualization (equal); writing – original draft (equal); writing – review and editing (equal). **Motohiro Ebisawa:** Conceptualization (equal); data curation (supporting); formal analysis (supporting); funding acquisition (lead); investigation (supporting); methodology (supporting); project administration (equal); resources (equal); software (supporting); supervision (lead); validation (lead); visualization (supporting); writing – original draft (equal); writing – review and editing (lead).

ACKNOWLEDGMENTS

We would like to thank Dr. Fukutomi and the entire staff of the Department of Pediatrics and Clinical Research Center for Allergy and Rheumatology, National Hospital Organization Sagami-hara National Hospital, and all children and parents who participated in this study. We would like to thank Editage (www.editage.com) for English language editing.

CONFLICT OF INTEREST

Motohiro Ebisawa and Sakura Sato have received lecture fees from Mylan EPD. All other authors declare that they have no relevant conflicts of interest.

PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/pai.13831>

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Takei M, Saito A, Yanagida N, Sato S, Ebisawa M. Cross-reactivity of each fraction among cereals in children with wheat allergy. *Pediatr Allergy Immunol*. 2022;33:e13831. doi: [10.1111/pai.13831](https://doi.org/10.1111/pai.13831)