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Original article

IgE antibodies to ω-5 gliadin associate with immediate symptoms on oral wheat challenge in Japanese children

Background: Gliadins have been implicated in immunoglobulin E (IgE)-mediated allergy to ingested wheat and ω-5-gliadin is known to represent a major allergen in wheat-dependent exercise-induced anaphylaxis. Less known is whether ω-5-gliadin is a clinically relevant allergen in children with immediate allergy to ingested wheat. This study investigates whether specific IgE antibodies to ω-5-gliadin (sIgE-ω-5-gliadin-ab) could be used as a marker for oral wheat challenge outcome in wheat-sensitized children. A secondary objective was to study whether the level of sIgE-ω-5-gliadin was related to symptom severity in children with a positive challenge test.
Methods: Serum samples from 88 children sensitized to wheat, of whom 35 underwent wheat challenge, were collected consecutively. sIgE-ω-5-gliadin-ab was related to a physician's diagnosis of wheat allergy and challenge symptoms.
Results: The mean concentration of sIgE-ω-5-gliadin-ab was 7.25 kU_A/l in patients with wheat allergy and 1.08 kU_A/l in patients with no wheat allergy ($P < 0.01$). sIgE-ω-5-gliadin-ab was only detected in 12 of the non-wheat allergic children and 11 of them had a specific IgE to wheat below 1.30 kU_A/l. Children reacting with severe symptoms upon challenge ($n = 8$) had increased levels of sIgE-ω-5-gliadin-ab compared to children with moderate, mild or no symptoms ($P < 0.001$).
Conclusions: The presence of sIgE-ω-5-gliadin-ab is related to the reaction level to wheat challenge outcome in wheat-sensitized children. The sIgE-ω-5-gliadin-ab was found to be associated with a strong convincing history of wheat allergy also in those cases when oral food challenge was avoided. The sIgE-ω-5-gliadin-ab level may serve as a marker for clinical reactivity in wheat-sensitized individuals.

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Food allergies account for 20% of acute urticaria, are present in 37% of children with moderate to severe atopic dermatitis and approximately 5% with atopic asthma and are the most frequent cause of anaphylaxis outside the hospital setting (1). Wheat is amongst the six foods responsible for approximately 90% of food allergies in children and is the third most common immediate type food allergens in Japanese children (2).

The most common presentations of food allergy are cutaneous followed by multiorgan reactions. Wheat is less likely to cause multiple organ system reaction on challenge when compared with peanut, milk and egg in sensitized individuals (3). Wheat-specific immunoglobulin E (IgE) concentrations do not seem to be such useful predictors of the challenge test outcome enabling reduction of these tests, in particular when compared with milk-, egg- and peanut-specific IgE concentrations (4). The frequently observed false-positive IgE reaction to wheat and other cereals in grass-pollen-allergic patients is explained by clinically insignificant cross-reactivity between the water/salt-soluble proteins (5). Therefore, identification of proteins associated with symptoms to wheat is of importance. Efforts have been made to identify new proteins that may be involved in food allergy to wheat (6–9). However, only a few of several wheat proteins recognized by the IgE of sensitized individuals have been characterized at the molecular level. Gliadins in particular have been implicated in IgE-mediated allergy to ingested wheat. They are seed storage proteins

Abbreviations: IgE, immunoglobulin E; kU_A/l, kilounits of allergen-specific IgE per litre; OFC, oral food challenge; DBPCFC, double-blinded placebo-controlled food challenge; SPT, skin prick test; WDEIA, wheat-dependent exercise-induced anaphylaxis; rω-5 gliadin, recombinant ω-5 gliadin; WA, child characterized with wheat allergy; NoWA, child without wheat allergy.

and members of the prolamin super family. They are characteristically soluble in aqueous alcohols (10). Gliadins trigger celiac disease but they are also major allergens in food allergy to wheat, capable of provoking IgE-mediated reactions.

Palosuo et al. (11) and Lehto et al. (12) identified ω-5-gliadin as an allergen in patients with wheat-dependent exercise-induced anaphylaxis (WDEIA). Similarly, Morita et al. (13) found that the studied WDEIA patients had IgE antibodies to fast ω-5-gliadin when tested by dot blotting.

Palosuo et al. (14) also found IgE antibodies to ω-5-gliadin in 16 of 19 children with atopic dermatitis who showed an immediate reaction to wheat, but they were not detected in children with delayed or no symptoms in oral wheat challenge. Armentia et al. (15) found that clinically significant reactivity to cereal may be observed in early life and the most important allergens were wheat followed by barley and rye. Although there are similarities in the possible adverse reactions to wheat, e.g. IgE-mediated food allergy, WDEIA, celiac disease, baker's asthma and non-IgE-mediated enteropathy, the clinical responses have not yet been completely resolved.

The primary objective of this study was to examine if recombinant ω-5-gliadin (rω-5-gliadin) could be used as a marker for oral wheat challenge outcome in wheat-sensitized children. A secondary objective was to study if the level of specific IgE antibodies to rω-5-gliadin was associated with symptom severity in children with a positive challenge test.

Materials and methods

Study population

Children sensitized to wheat (median: 12.5 kU_A/l; range: 0.70–100 kU_A/l), with symptoms from the skin and/or respiratory and gastrointestinal tract, and referred to Aichi Children's Health and Medical Center, were consecutively included in the study. In total, 88 children were included. At study entry each patient was clinically evaluated, with focus on wheat allergy, by both a detailed medical examination and collection of their medical history. A new blood sample was also taken for baseline determination of specific IgE antibody to wheat and ω-5-gliadin. In 35 uncertain cases an oral wheat challenge test had to be performed in order to confirm or exclude wheat allergy. The reason why challenge was not performed in the remaining 53 children was due to either a convincing negative history or a convincing positive history with high risk of a strong reaction on challenge. Based on case history, physical examination, laboratory tests and, in some children, the challenge outcome, each child was eventually classified to have an IgE-mediated immediate hypersensitivity to ingested wheat (designated as wheat allergy, or WA) or not (designated no wheat allergy, or NoWA).

Laboratory studies

Serum samples were analysed for wheat and rω-5-gliadin allergen-specific IgE antibodies using the ImmunoCAP® System FEIA (Phadia AB, Uppsala, Sweden). The rω-5-gliadin was prepared as previously described (16).

Oral food challenge

All wheat-food challenges were open challenges, performed in hospital settings and supervised by physicians in accordance with the guidelines for diagnosis and management of paediatric food allergy in Japan (17). During the challenge, full emergency equipment was at hand. Informed consent was given by the children's parents prior to enrolment in the study.

The child was given udon noodles (containing wheat flour, water and salt) in stepwise increasing amounts (trace amount, 1, 2, 5, 10 and 20–50 g). A child's maximal dose was established according to the child's age and prior knowledge of the severity of allergic reaction. As udon noodles contain 2.6% wheat protein, the amount of the challenged dose was equivalent in the range 26 mg to 1.3 g wheat protein. In general, the time interval between doses was 20 min but could be prolonged if the patient claimed suspicious symptoms.

A challenge was scored as positive if one or more of the following objective clinical reactions was observed: (i) cutaneous symptoms including a pruritic, erythematous morbilliform rash or urticaria; (ii) coughing and laryngeal oedema (upper respiratory symptoms), wheeze and dyspnoea (lower respiratory symptoms); and (iii) gastrointestinal symptoms including nausea, abdominal pain, vomiting and diarrhoea. Furthermore, the symptoms were classified according to severity of the reaction, i.e. (i) mild (localized cutaneous symptoms or mild upper respiratory symptoms only), (ii) moderate (general cutaneous symptoms, upper respiratory and/or gastrointestinal symptoms) and (iii) severe (lower respiratory symptoms and/or collapse).

In 14 of the 35 patients that underwent challenge, no reaction was observed at all. Table 1 gives the individual outcomes from the challenge procedure for each of the 21 patients that showed a positive reaction.

Statistical methods

The Kruskal–Wallis test was used to test differences between groups. A *P*-value of 0.05 was judged as significant. Performance characteristics, i.e. sensitivity and specificity, were calculated for various cut-off values, including the optimal cut-off values proposed by the receiver operating characteristics (ROC) plots. Statistical analysis was carried out using SAS System V8.2 (SAS Institute Inc, Cary, NC, USA).

Results

Oral wheat challenge outcome and demographics

Of the 35 children who underwent challenge, 21 were found to be positive (Table 1). Five children reacted with mild symptoms, eight with moderate and eight with severe. Lower respiratory symptoms were seen in all of the eight children with severe symptoms. Upper respiratory symptoms were observed in five of the children with moderate symptoms.

Twenty-three children had a strong convincing history of wheat allergy and thus challenges were not performed because of the high risk for anaphylaxis. These children had all experienced a recent severe reaction and were regarded as highly likely to react strongly to a wheat challenge. Thirty children had a negative history and thus

Table 1. Individual outcomes of the challenge procedure for each of the 21 patients

Patient	Wheat allergy-related symptoms	Intensity of reaction at challenge	Amount of udon noodle given (g)
1	Urticaria	Moderate	8
2	Cough, skin redness	Moderate	8
3	Eczema	Mild	1
4	Vomiting, wheeze, skin reaction	Severe	0.1
5	Urticaria	Mild	8
6	Throat itching, stomach ache, skin redness	Moderate	2
7	Anaphylaxis	Severe	5
8	Anaphylaxis	Severe	3
9	Quincke's oedema	Mild	18
10	Cough, OAS	Mild	36
11	Anaphylaxis	Severe	1
12	Cough	Mild	5
13	Cough, wheeze, skin redness	Severe	8
14	Face redness	Mild	36
15	Urticaria, running nose, throat itching	Moderate	38
16	Oedema, skin redness	Moderate	68
17	Urticaria	Moderate	8
18	Skin redness	Moderate	8
19	Cough, wheeze, urticaria	Severe	48
20	Wheeze, skin redness	Severe	11
21	Wheeze, systemic urticaria	Severe	18

excluded from challenge because of the unlikelihood of wheat allergy. Thus, the clinical histories of 44 children were suggestive of WA and the remaining children ($n = 44$) were NoWA.

In the study, the WA group comprised 30 males and 14 females (32%). Corresponding figures for the NoWA group were 31 and 13 (30%) respectively. Mean age in the WA group was 3.4 years (range 1–8.7 years). Corresponding mean age in the NoWA group was 4.9 years (1–15).

Allergen-specific IgE

The median concentrations of wheat-specific IgE antibodies were 33.8 (11.5–84.1, 25–75% observation) kU_A/l in the WA group and 7.2 (5.0–18.1) kU_A/l in the NoWA group ($P < 0.01$, Fig. 1). The median concentrations of specific IgE antibodies to rω-5-gliadin were 2.45 (0.35–5.77, 25–75% observation) kU_A/l in the WA group and 0.35 (0.35–0.37) kU_A/l in the NoWA group ($P < 0.01$, Fig. 1). In the WA group, the concentration of specific IgE antibodies to rω-5-gliadin was >0.35 kU_A/l in 37 of 44 (84%) of the cases, but only 12 of 44 (27%) in the NoWA group.

The area under the ROC curve for rω-5-gliadin was 85.7% (Fig. 2) and for the estimated cut-off of 0.43 kU_A/l a value of 82% was obtained for both sensitivity and specificity for the assay. Corresponding sensitivity and specificity at the cut-off of 0.35 kU_A/l was 84% and 73% respectively. The area under the ROC curve for wheat was 76.9% (Fig. 2) and for the estimated cut-off of 12.5 kU_A/l a value of 75% and 70% was obtained for the sensitivity and specificity, respectively, for the assay. The mean concentration of specific IgE antibodies to rω-5-gliadin in the WA group was 7.25 kU_A/l (range, 0.40–81.0 kU_A/l) and 1.08 kU_A/l (<0.35–4.80) in the NoWA group ($P < 0.01$). rω-5-gliadin was detected in 12 children in the NoWA group and, of these, 11 children were found in the range 0.35–1.30 kU_A/l.

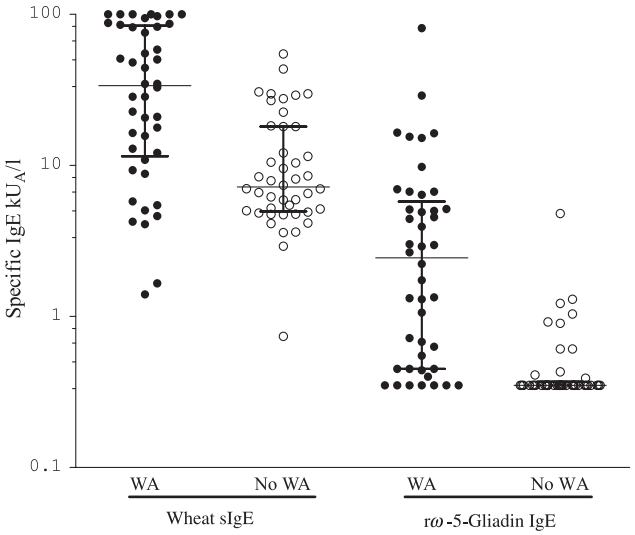

Figure 1. Doctor's diagnosis of wheat allergy (WAvs NoWA) and levels of specific IgE to wheat and to rω-5-gliadin. Whiskers include the interquatile range (25%–75% observation).

The patients were divided into two groups according to the procedure of diagnosis, i.e. diagnosis by challenge test or convincing history (Fig. 3). The children with a positive challenge had significantly higher concentrations of specific IgE antibodies to rω-5-gliadin compared with those patients that had a negative challenge ($P < 0.01$, Fig. 3). The same was true for the children with a positive

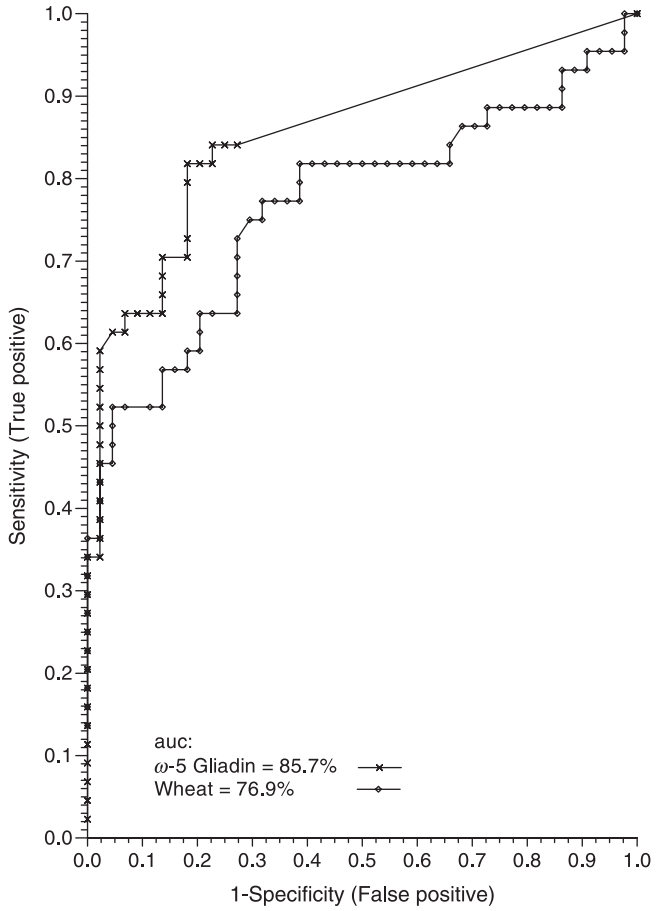

Figure 2. ROC curve based on all patients (pos = wheat allergy patients, $n = 44$; neg = controls, $n = 44$) (auc = area under the curve).

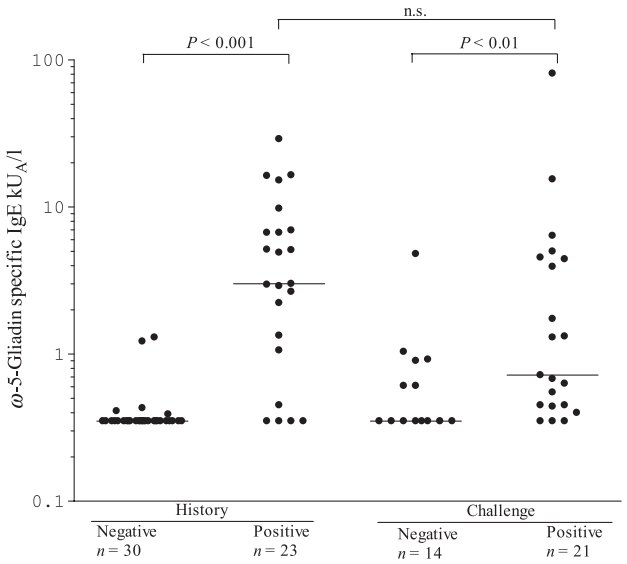

Figure 3. Levels of specific IgE to rω-5 gliadin (median) in children where clinical history was used to diagnose wheat allergy or no wheat allergy. Also levels of specific IgE to rω-5 gliadin are shown in children that underwent challenge with wheat containing udon noodles to determine wheat allergy or no wheat allergy.

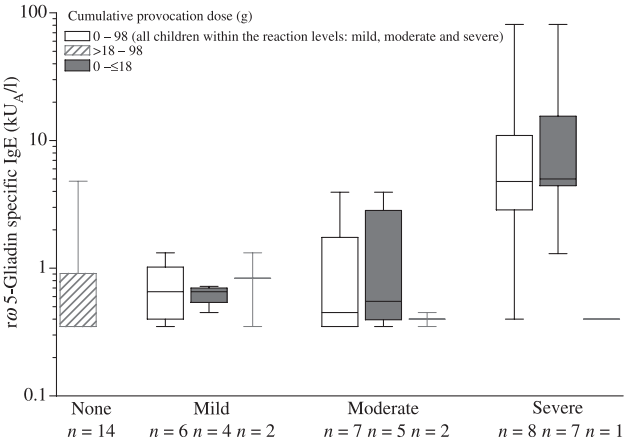

Figure 4. ω-5- gliadin levels in 4 different reaction levels at challenge with wheat containing udon noodles. Boxes include the interquatile range (25%–75% observation). Whiskers extend to the most extreme data point.

convincing history compared to the children with a negative history ($P < 0.001$, Fig. 3). There was no statistical difference in specific IgE titres to rω-5-gliadin between the challenge positive and the history positive children.

Further analysis performed in the challenge positive patients showed that children with severe reactions had significantly higher concentrations of specific IgE antibodies to rω-5-gliadin compared to children with mild or moderate symptoms ($P < 0.001$).

On the basis of the 35 observations from the challenge procedure, the relationships between reaction levels at challenge (none, mild, moderate and severe) and levels of specific IgE against rω-5-gliadin are shown in Fig. 4. Furthermore, the children were grouped into one of the two groups depending on the amount of wheat-containing udon noodles given before a reaction could be observed. In 14 cases there was no reaction at all and these patients were also classified as non-wheat allergics. In cases where reaction could be observed at provocation doses of 0.1–18 g of noodles, the levels of specific IgE against rω-5-gliadin antibodies were higher than when a reaction could be observed at provocation doses of 19–100 g of noodles (Fig. 4).

Discussion

This study shows that the presence of ω-5-gliadin-specific IgE antibodies is related to the immediate symptoms after ingestion of wheat in wheat-sensitized children. Furthermore, ω-5-gliadin-specific IgE antibodies were found to be associated with a strong convincing history of wheat

allergy in those cases when oral food challenge was avoided due to the risk for anaphylaxis. The ω-5-gliadin-specific IgE antibody level may thus serve as a risk marker for clinical reactivity.

Oral challenge test is the gold standard for the diagnosis of food allergy, but it sometimes causes severe reactions, including anaphylaxis. Few medical interventions are entirely free of risk and most clinicians would hesitate to challenge children in general who have a recent history of severe reaction, very high serum IgE or those with severe or unstable asthma. We have shown in this study that patients with a high concentration of IgE to ω-5-gliadin had a high risk of failing a wheat challenge. Furthermore, the IgE concentration correlated with severe symptoms and the threshold amount of wheat allergen provoking symptoms in the challenge test. It is possible to define threshold levels for predicting a positive reaction after oral challenge using ω-5-gliadin. We did not, however, calculate these levels in this paper due to an insufficient number of wheat-challenged children. Increased usage of ω-5-gliadin tests will provide sufficient data for these probability calculations. The challenge test might therefore, in the future, be avoided in children with high levels of IgE to ω-5-gliadin, according to our findings as well as those made by Palosuo et al. (14). We found a strong difference in ω-5-gliadin-specific IgE in children with severe symptoms in comparison with non-symptomatic children and children with mild to moderate symptoms. These latter groups seem to have similar levels when the challenge dose is taken into account. Further studies are needed to verify our findings but it seems that specific IgE to rω-5-gliadin can be useful as a marker for severe reactions.

Palosuo et al. (14) have previously reported that detection of IgE antibodies to purified ω-5-gliadin using their ELISA system offered 100% specificity and 84% sensitivity for the diagnosis of immediate type wheat allergy. We have, in our study, calculated somewhat lower specificity and sensitivity. However, Palosuo et al. recruited a relatively small number of wheat-sensitized patients in the challenge negative and control groups resulting in 100% specificity. The advantage of our study was the high number of wheat-sensitized children included in the no wheat allergic group.

One limitation of our study is the retrospective design. The optimal way to investigate how allergen-specific IgE levels are related to a clinical course of food allergy is to conduct a prospective study. This is, however, both time and cost-consuming and ω-5-gliadin-specific IgE antibody testing has only recently become commercially available. Another limitation of the study is that some wheat-allergic patients without specific IgE antibodies to wheat but with specific IgE antibodies to ω-5-gliadin may have been missed.

IgE levels have been regarded as a useful predictor of food challenge outcome but a less useful predictor for the degree of reaction severity during failed food challenge (18). van Odijk et al. have shown that peanut-sensitized individuals who report the worst reaction after ingestion had higher levels of specific IgE (19), even if this was not based on challenge data. Similarly, Benhamou et al. showed recently that specific IgE antibody levels for egg white correlated with the severity of reactions in egg allergic patients at challenge (20). Hourihane et al. have shown that peanut-specific IgE antibody concentration can predict severity of reaction in DBPCFC, if the dose of allergen is considered (21). These are similar findings to ours when the threshold dose is taken into consideration but needs to be further verified in larger study groups.

There are few other clinical biomarkers available for the diagnosis of severity of the symptoms and threshold level of allergens that provoke allergic reactions. Our studied protein may offer a unique model of food allergen, where one allergen component in the food contributes to a special allergic symptom, whilst other components, like water/salt-soluble proteins such as peroxidase (22), serpin (23) and sub-units of α-amylase inhibitor and recently also LTP (24), seem to be mainly associated with symptoms like baker's asthma. However, recently other proteins that may be useful in the diagnosis of wheat allergy have also been identified (6, 9). One example is the high-molecular-weight glutenin (HMW) that is associated with WDEIA symptoms in patients that do not have IgE antibodies to ω-5-gliadin (25–27). In addition, other candidate wheat proteins (i.e. LTP, etc.) may be considered of interest in the diagnosis of wheat allergy but further investigations are needed in order to find out if there are associations with specific symptoms (6). The involvement of ω-5-gliadin in severe reactions may be due to the structure of the protein, which consists mostly of glutamine. Primary sequences of IgE-binding epitopes have been determined in WDEIA patients (8, 25), which included proline residues in the glutamine background. This characteristic contributes to the water/salt-insoluble nature of the protein so that it is hardly absorbed from the mucosal membrane of the oral or pharyngeal cavity. Palosuo (28) also reported on the role of tissue transglutaminase, which cross-links the pepsin-digested ω-5-gliadin peptides and increases the IgE binding capacity. Taken together, this suggests that the sensitization route may be through the small intestine rather than through the oral, pharyngeal or upper respiratory tracts. In contrast, the symptoms are displayed rather quickly and, in some cases, systemically. This could be due to the biochemical properties of omega gliadins, in that they lack cysteins and are therefore unable to be included in the network of glutenins and gliadins formed during the baking process (29). The ω gliadins are, in this respect, more easily dissolved and thereby more accessible than other proteins in the baked food. To fully understand the relationship between the IgE sensitization to ω-5 gliadin and the nature of the symptoms further studies have to be performed that address these particular questions.

We conclude that detection of IgE to rω-5 gliadin seems to be associated with failed challenge outcome and

may serve as a good aid for deciding if challenge should be performed or not.

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