



# NEW HORIZONS — ALLERGY —

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## Diagnostic test using recombinant omega-5 gliadin for wheat-dependent, exercise-induced anaphylaxis

### Summary

Food-dependent, exercise-induced anaphylaxis (FDEIA) is a distinct form of food allergy induced by physical exercise. Symptoms are typically generalized urticaria and severe allergic reactions such as shock or hypotension. Whereas numerous food items are responsible for the development of FDEIA, wheat is reported to be the allergen with the highest frequency in Japan. Skin tests and *in vitro* serum food-specific IgE assays are currently used for diagnosis but their sensitivity and specificity are not always satisfactory.

A challenge test consisting of ingestion of the suspected food followed by intense physical exercise is the only reliable method of determining the causative food and of diagnosing the disease. The challenge test is, however, inconvenient and even dangerous because in some cases it induces an anaphylactic shock.

A reliable *in vitro* diagnostic method is thus required for patients with FDEIA. We revealed that wheat omega-5 gliadin and high molecular weight glutenin (HMW-glutenin) are major allergens in wheat-dependent, exercise-induced anaphylaxis (WDEIA). A simultaneous detection of IgE specific to epitope sequences of both omega-5 gliadin and HMW-glutenin using ImmunoCAP™ is found to achieve higher sensitivity and specificity compared with the *in vitro* serum food-specific IgE assays currently used to diagnose WDEIA.

In addition, the recombinant omega-5 gliadin ImmunoCAP allergen contains the immuno-dominant peptides since the results correlated well with the IgE antibody binding to the identified major epitope peptides in omega-5 gliadin used to identify patients with WDEIA and wheat allergy.

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### Keywords

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High molecular weight glutenin,  
wheat allergy.

### Introduction

Nowadays, IgE-mediated hypersensitivity to foods is clearly recognized as food allergy. The allergic reaction usually appears after ingestion of allergenic food. However, a number of cases show anaphylactic reaction only during or in association with exercise after ingestion of foods. This clinical entity was termed food-dependent, exercise-induced anaphylaxis (FDEIA). The anaphylactic symptoms are usually induced by physical exercise after food ingestion and approximately 80% of patients display symptoms within 2 hours after eating. The symptoms include urticaria, angioedema, respiratory symptoms, abdominal pain, fatigue and loss of consciousness. Numerous episodes of these symptoms are commonplace.

In many cases, strenuous exercise, such as running or playing tennis, triggers anaphylaxis after ingesting specific food(s) [1-3]. However, even milder exercise often induces symptoms. Additional triggering factors in FDEIA have been

reported to include general physical conditions, drugs, alcohol and atmospheric conditions. Induction of anaphylaxis has been clearly demonstrated to be dependent on the amount of allergen ingested in patients with wheat-dependent, exercise-induced anaphylaxis (WDEIA) [4]. Aspirin intake has been well documented to induce symptoms, or to provoke more severe symptoms compared with symptoms without aspirin intake [5-7]. Aspirin may also induce symptoms in combination with food ingestion, even without exercise challenge [6, 7].

Currently, the useful diagnostic tests for food allergy are skin tests, *in vitro* serum food-specific IgE assays, and oral food challenges. Skin tests (a skin prick test is usually performed) and *in vitro* serum food-specific IgE assays do provide some important information. However, a positive reaction in skin prick testing or a positive value in *in vitro* serum food-specific IgE assays does not necessarily indicate

the causative foods. ImmunoCAP™ is an accurate and standardized procedure for determining allergen-specific IgE in sera and is now widely used for diagnosing food allergy. The utility of food-specific IgE concentrations in predicting clinical reactivity has been reported in egg, milk, peanut and fish allergy [8]. In cereal allergy, especially WDEIA, this is not always the case. Wheat and gluten-specific IgE measurement is available for diagnosing WDEIA with ImmunoCAP allergen, but approximately 30% of patients with definite WDEIA are negative for wheat or gluten [9]. In addition, a considerable number of patients with atopic dermatitis have positive ImmunoCAP results for wheat and/or gluten, yet have no episode of IgE-mediated allergic reactions after ingesting wheat products. Thus, the measurement of food-specific IgE antibodies using wheat ImmunoCAP is not always satisfactory for diagnosing FDEIA.

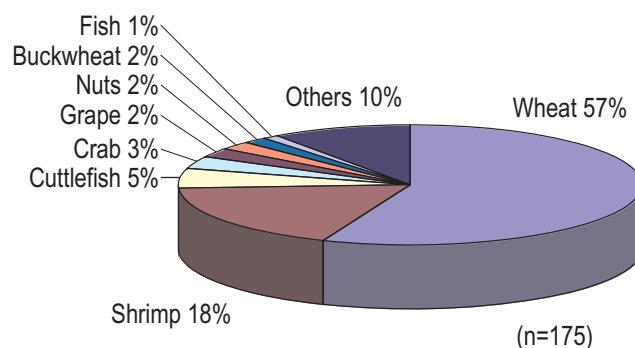
### Challenge tests

Challenge tests will give a definite diagnosis of FDEIA in patients with a history of occasional anaphylaxis after food ingestion. If a definite food has not been identified as the cause of the reaction, a food challenge test is necessary because correct identification of the causative food might be life-saving. Since food challenge alone usually does not elicit anaphylactic symptoms, food ingestion combined with exercise is needed to confirm the causative food as well as the diagnosis [5, 10]. The challenge includes food challenge alone, exercise alone, and a combination of food ingestion and exercise. The exercise challenge has been performed according to the protocol of Bruce that has been used for diagnosing ischemic heart disease. However, intake of a specific food followed by exercise does not always provoke the anaphylactic symptoms. Challenge tests can confirm the diagnosis of FDEIA only in up to 70% of patients, even in patients with histories of recurrent episodes of anaphylaxis during exercise after ingestion of specific foods [5, 10]. Induction of anaphylactic symptoms was documented to be dependent on the amount of food allergen ingested [4], indicating that insufficient amounts of food allergen ingested may cause cases of false-negative clinical response in challenge testing. Another possible factor for causing false-negative results is inappropriate exercise employed in the tests. Some other conditions such as fatigue, lack of sleep, or cold environments are considered to affect the development of the anaphylactic symptoms.

As aspirin intake has been well documented to provoke more severe symptoms in several patients [5-7, 10], food challenge with aspirin-pretreatment has been performed for diagnosis. In fact, a number of patients elicited anaphylactic symptoms by a challenge with just a combination of food and aspirin, even without exercise and despite them never having experienced anaphylactic symptoms induced by aspirin or non-steroidal, anti-inflammatory drugs.

### Determination of wheat allergens and IgE-binding epitopes

A variety of foods have been described as causal foods in FDEIA. These include shellfish [1, 5, 6, 11, 12], wheat products [3-7, 11,13,14], vegetables [2, 10, 11, 15], fruits [5, 11, 16], nuts [11], egg [11, 17], mushrooms [18], corn [11, 19], garlic [11], pork/beef [11], and rice [11]. In European countries, vegetables are common food allergens causing FDEIA [11]. However, in Japan, wheat is the most frequent allergenic food causing FDEIA [20]. Among 175 reported Japanese cases, approximately 60% were found to be wheat-induced (**Fig. 1**).



**Figure 1.** Frequency of causative foods in Japanese patients with FDEIA.

Wheat is composed of four classes of protein; albumins, globulins, gliadins, and glutenins. Albumins and globulins are water/salt soluble proteins, while gliadins and glutenins are water/salt insoluble proteins termed gluten [21]. Wheat allergens have been mainly analyzed in baker's asthma, a hypersensitivity to inhaled wheat flour, and several antigens were identified in both the water/salt soluble and insoluble proteins [22, 23]. Wheat allergens in FDEIA have been rather controversial. Wheat gliadins, which are major components of the gluten fraction, were found to be the allergens in WDEIA using skin testing and immunoblotting [24-26] studies. Recently, omega-5 gliadin has been identified as a cause of WDEIA [27, 28]. An analysis with a panel of purified gliadins and glutenins revealed that 80% of patients with WDEIA react most strongly to omega-5 gliadin, and the rest most strongly to high molecular weight glutenin (HMW-glutenin) [29]. When IgE-binding epitopes within the primary sequence of omega-5 gliadin were analyzed using arrays of overlapping peptides, seven epitopes (QQIPQQQ, QQLPQQQ, QQFPQQQ, QQSPEQQ, QQSPQQQ, QQYPQQQ and PYPP) were detected [29]. By using sera of 15 patients, four of these peptides (QQIPQQQ, QQFPQQQ, QQSPEQQ and QQSPQQQ) were found to be dominant epitopes. Mutational analysis of QQIPQQQ and QQFPQQQ indicated that amino acids at positions 1(Q), 4(P), 5(Q), 6(Q) and 7(Q) were critical for IgE antibody-binding [29]. IgE antibody-binding epitopes in the HMW-glutenin were identified to be QQPGQ, QQPGQGQQ and QQSGQGQ using the same method [9].

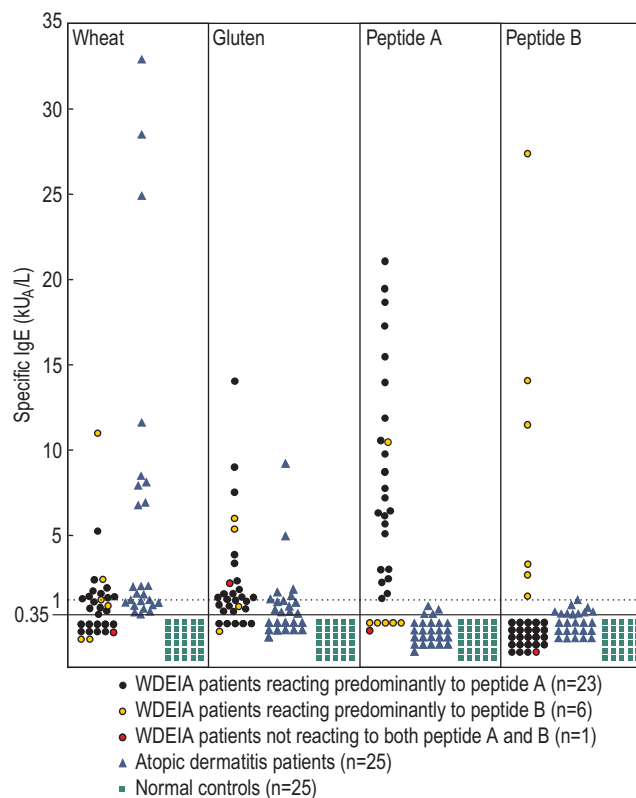
## Detection of epitope-specific IgE in the sera of the patients with WDEIA

When the IgE antibody-binding epitopes of omega-5 gliadin (peptide A) and HMW-glutenin (peptide B) were conjugated to ImmunoCAP to determine the specific IgE antibodies to these epitope peptides, 97% (29/30) of the patients with WDEIA were found to be positive to either of the two, whereas gluten-ImmunoCAP positively recognized only 80% (24 of 30) of the patients (Fig. 2) [9]. In addition, specific IgE antibody values using epitope peptide-ImmunoCAP were much higher than the gluten-specific test in most patients with WDEIA, indicating higher sensitivity with the epitope system. This is due to a higher content of epitope sequences in the epitope-peptide ImmunoCAP than in the gluten ImmunoCAP. Ten of 25 patients with atopic dermatitis who had no obvious allergic reactions after ingesting wheat products had a positive ImmunoCAP for epitope peptides, indicating the low specificity of the test. When the positive cut-off value was set at 1.0 kU<sub>A</sub>/L, both the sensitivity and specificity of the epitope peptide-specific CAP test were satisfactory. Sensitivity reached 97% (29/30) and specificity 98% (1/50) [9].

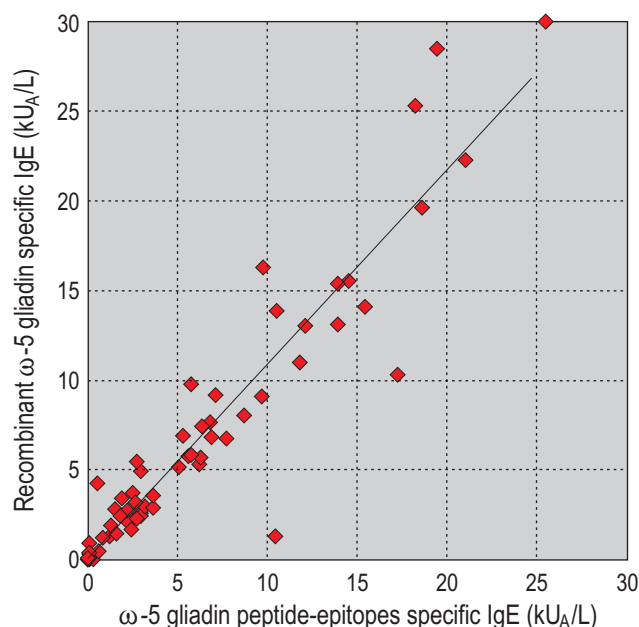
## Detection of recombinant omega-5 gliadin-specific IgE

Recently, recombinant food allergens of constant quality and quantity have been produced and tested in the diagnosis of many food allergies [30-32]. ImmunoCAP conjugated with recombinant omega-5 gliadin proteins was found to be a useful tool. We cloned a omega-5 gliadin gene that encodes a 439 amino acid-long protein with a calculated mass of 53 kDa and, by means of the expression system in *Escherichia coli*, constructed a recombinant C-terminal half-protein (178 amino acids) of the omega-5 gliadin that included all IgE antibody-binding epitopes [32]. The recombinant omega-5 gliadin was found to have an IgE antibody-binding ability comparable to natural omega-5 gliadin. When the levels of specific IgE antibodies to recombinant omega-5 gliadin were measured with ImmunoCAP in 55 patients with WDEIA, the value was well correlated with those of specific IgE antibody to the synthetic peptide epitopes of omega-5 gliadin conjugated to ImmunoCAP (Fig. 3).

The usefulness of ImmunoCAP with recombinant omega-5 gliadin was also confirmed when identifying children having severe allergic reactions to wheat. When 30 children having high serum titers of wheat-specific IgE antibodies (14 positive in wheat challenge test, the rest negative) were investigated with ImmunoCAP with recombinant omega-5 gliadin, 11 of the 14 with a positive provocation test had considerable levels of IgE antibodies to recombinant omega-5 gliadin (Dahlström J. *et al.*, submitted). In contrast, only 3 of the 16 children with a negative provocation test had detectable IgE antibody levels to omega-5 gliadin, indicating that measurement of IgE antibodies to recombinant omega-5 gliadin is a very good tool to identify children at risk of having severe immediate reactions to wheat.



**Figure 2.** Detection of specific IgE to wheat, gluten and epitope peptides of omega-5 gliadin (peptide A) and HMW-glutenin (peptide B) in WDEIA.



**Figure 3.** Correlation between IgE antibodies to recombinant omega-5 gliadin ImmunoCAP™ allergen and omega-5 gliadin peptide-epitope-conjugated ImmunoCAP using sera from patients with WDEIA.

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