

BRIEF COMMUNICATION

IgE detection to $\alpha/\beta/\gamma$ -gliadin and its clinical relevance in wheat-dependent exercise-induced anaphylaxis

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Abstract

Wheat-dependent exercise-induced anaphylaxis (WDEIA) is characterized by anaphylactic reactions after wheat ingestion and physical exercise. IgE antibodies to recombinant ω_5 -gliadin are detectable in a majority of WDEIA patients, but other wheat allergens may also play a role in elicitation of WDEIA. Here, we performed a comprehensive analysis of IgE reactivity to different wheat proteins in 17 patients with confirmed WDEIA by ImmunoCAP research prototypes and a semi-quantitative microarray immunoassay with $\alpha/\beta/\gamma$ -gliadin, high-molecular-weight (HMW) glutenin, alpha-amylase inhibitor (AAI) dimer, and wheat lipid transfer protein (LTP). By ImmunoCAP, IgE to recombinant ω_5 -gliadin was detectable in 14/17 patients (82%), to $\alpha/\beta/\gamma$ -gliadin in 82% including the three patients lacking IgE to ω_5 -gliadin, and to HMW glutenin in 59%. The microarray revealed specifically γ -gliadin as the second most important allergen. These results demonstrate the additional diagnostic value of α/β - and γ -gliadin in particular in ω_5 -gliadin-negative patients in the diagnosis of WDEIA.

Wheat-dependent exercise-induced anaphylaxis (WDEIA) is a rare, but potentially severe form of food allergy (1, 2). It is clinically characterized by anaphylactic reactions ranging from urticaria to respiratory, gastrointestinal, or cardiovascular symptoms that occur usually 1–4 h after wheat ingestion followed by physical exercise. Cofactors such as aspirin, alcohol intake, infections, stress, or female sex hormones/

menstruation can substitute for or synergize with exercise in eliciting the immediate-type hypersensitivity reaction after ingestion of wheat (3).

Wheat proteins are classified into water/salt-soluble proteins (e.g., alpha-amylase inhibitor) and water/salt-insoluble gluteins. The latter group comprises gliadins (classified into α/β -, γ -, and ω -gliadins according to their electrophoretic

mobility) and glutenins. Specifically, ω_5 -gliadin (Tri a 19) has been described as a major allergen, and specific IgE antibodies to recombinant ω_5 -gliadin are detectable by ImmunoCAP (Phadia AB, Uppsala, Sweden) in approximately 80% of WDEIA patients (4). In WDEIA, sensitization to wheat proteins has been suggested to occur mostly intestinal. More recently, rhinoconjunctival or cutaneous contact of wheat protein hydrolysates present in cosmetic products has been described as route of sensitization (5). In patients sensitized to hydrolyzed wheat proteins, ω_5 -gliadin seems not to be the major allergen, instead other gliadins or glutenins were suggested to be relevant (5).

Here, we assessed the IgE profile to a broad spectrum of well-characterized and highly purified wheat allergens in 17 patients with conventional WDEIA (Table 1). The diagnosis was based on a typical history in all patients: a combination of wheat intake and exercise as eliciting factors was reported by 12/17 patients, while in three patients, additional factors (aspirin, alcohol, or infection) were required, and in two patients, aspirin or alcohol intake provoked WDEIA also in the absence of exercise (patient no. 6 and 10). The diagnosis was confirmed by a positive food and exercise challenge test following wheat ingestion in 10/12 patients (five patients declined a challenge test). The initial diagnostic work-up also included skin prick tests using commercial wheat extracts (positive in 29%) and native wheat flour (positive in 80% of patients), determination of total IgE levels (elevated in 82%), and the detection of IgE to wheat extract (f4) (positive in 59%) and to recombinant ω_5 -gliadin (f416) that was positive in all but three patients (82%).

To identify additional wheat allergens putatively responsible for WDEIA, IgE levels to different native or recombinant wheat allergens were analyzed by using experimental ImmunoCAP tests with $\alpha/\beta/\gamma$ -gliadin, high-molecular-weight (HMW) glutenin, an alpha-amylase inhibitor (AAI) dimer, and wheat lipid transfer protein (LTP) (Fig. 1). Positive IgE reactivity (≥ 0.35 kU_A/l) to $\alpha/\beta/\gamma$ -gliadin was identified in 14/17 (82%) patients, including the three patients lacking IgE to ω_5 -gliadin. IgE to HMW glutenin was detected in 10/17 patients (59%), however not in the three ω_5 -gliadin-negative patients. Only one serum reacted with AAI dimer and wheat LTP.

To confirm these results and for a more detailed study of IgE reactivity to wheat gliadins, an experimental semi-quantitative microarray immunoassay was employed (6) using highly purified native α/β -gliadin, γ -gliadin, AAI dimer 0.19, and wheat LTP.

This microarray revealed IgE reactivity to recombinant ω_5 -gliadin in 15/17 (88%), to α/β -gliadin in 9/17 (53%), to γ -gliadin in 13/17 (76%), to AAI dimer 0.19 in 4/17 (23.5%), and to wheat LTP in 1/17 patients (6%). Interestingly, in one patient with WDEIA confirmed by provocation test and IgE antibodies to $\alpha/\beta/\gamma$ -gliadin by ImmunoCAP, the microarray identified γ -gliadin as the only IgE-reactive allergen.

None of the 20 nonatopic controls reacted with any of the allergens by ImmunoCAP or microarray. In addition, none of the 21 food allergic patients used as controls (with sensitization to tree nuts, fish, shrimps, sorghum, sesame, vegetables, or pollen-associated food allergens) displayed IgE antibodies to $\alpha/\beta/\gamma$ -gliadin and only one patient to HMW

glutenin by ImmunoCAP (Fig. 1). Similarly, one food allergic control individual reacted with γ -gliadin by the microarray, none with α/β -gliadin or HMW glutenin, indicating specificity for the detection of IgE antibodies to α/β - or γ -gliadin and HMW glutenin of at least 97.5%.

Gliadins and glutenins have been identified as causative allergens in WDEIA. Our data corroborate the importance of the ω_5 -gliadin ImmunoCAP test for the *in vitro* diagnosis of WDEIA allowing us to confirm the diagnosis in 82% of our patients (4). HMW glutenin was described as the second major allergen in WDEIA, and 97% of Japanese WDEIA patients could be diagnosed by determination of specific IgE to ω_5 -gliadin and HMW glutenin (7). Although HMW glutenin was targeted by IgE antibodies in 59% of our patients (by ImmunoCAP), none of the three ω_5 -gliadin-negative individuals showed reactivity to this allergen. In contrast, all three patients lacking reactivity to ω_5 -gliadin displayed specific IgE against $\alpha/\beta/\gamma$ -gliadin by ImmunoCAP. Thus, in our patient population, α/β - or γ -gliadins appeared to be of higher relevance as IgE-reactive proteins than HMW glutenin. A role for α/β - or γ -gliadins as allergens in wheat allergic patients and in some cases of WDEIA has been suggested previously (8, 9). Our detailed analysis by microarray indicated that γ -gliadin was the only IgE-reactive gliadin in two of three ω_5 -gliadin-negative patients. One of them (no. 2) did not display IgE to any other wheat allergen by ImmunoCAP and microarray, suggesting that γ -gliadin alone may elicit WDEIA. For this patient, WDEIA was confirmed twice by oral provocation including exercise and aspirin as augmentation factors. The second patient (no. 15) had a history of baker's asthma and WDEIA and reacted with γ -gliadin, AAI dimer, and wheat LTP. The third patient (no. 16) displayed pronounced IgE signals to α/β -gliadin and low signals to ω_5 -gliadin and γ -gliadin in the microarray.

To our knowledge, this is the most comprehensive analysis of IgE reactivity to different wheat allergens in patients with WDEIA on a widely available assay platform (ImmunoCAP). This analysis indicates a high prevalence of specific IgE not only to ω_5 -gliadin but also to HMW glutenins and α/β - and γ -gliadin, which are of additional diagnostic value in particular in ω_5 -gliadin-negative patients. The analysis of these parameters by reliable and commercially available assays will help us to close the gap in the *in vitro* diagnostics of WDEIA.

Acknowledgments

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Conflict of interest

TJ and TB have received research grants, honoraria and consulting fees from Phadia, Germany, now Thermo Fisher Scientific, Germany. CE and OBG are employees of Thermo Fisher Scientific, Uppsala, Sweden, and have otherwise no conflict of interest to disclose. SCH and JF have no conflict of interest to disclose.

Table 1 Summary of clinical characteristics, serological data, and results of food and exercise challenge tests of wheat-dependent exercise-induced anaphylaxis (WDEIA) patients

Clinical data			ImmunoCAP (kU _A /l)				Microarray*							
Patient No./sex/age	Max. grade of anaphylaxis†	Provocation	Total IgE	Wheat (f4)	ω ₅ -gliadin (f416)	α/β/γ-gliadin	HMW glutenin	AAI dimer 0.19	Wheat LTP	ω ₅ -gliadin	α/β-gliadin	γ-gliadin	AAI dimer 0.19	Wheat LTP
1/f/44	3	+	385.0	1.70	6.40	8.12	2.92	<0.35	<0.35	2	2	3	1	0
2/m/55	1	+	39.5	<0.35	<0.35	0.60	<0.35	<0.35	<0.35	0	0	1	0	0
3/f/47	3	+	1303.0	0.69	10.6	4.33	1.28	<0.35	<0.35	3	1	2	0	0
4/m/37	1	+	126.0	0.74	10.0	1.98	0.78	<0.35	<0.35	3	1	2	0	0
5/m/31	2	+	421.0	0.79	9.2	0.60	0.67	<0.35	<0.35	3	0	2	0	0
6/f/29	2	+	214.0	<0.35	1.8	<0.35	<0.35	<0.35	<0.35	2	0	0	0	0
7/m/29	3	+	187.0	<0.35	6.2	0.38	0.38	<0.35	<0.35	3	0	1	0	0
8/m/60	3	ND	64.7	0.36	3.8	0.38	<0.35	<0.35	<0.35	2	0	0	0	0
9/m/38	2	–	480.0	14.5	13.9	16.79	0.81	<0.35	<0.35	3	3	3	1	0
10/m/38	2	+	60.4	<0.35	3.10	<0.35	<0.35	<0.35	<0.35	2	0	0	0	0
11/f/34	3	ND	700.0	<0.35	21.2	1.44	0.88	<0.35	<0.35	3	1	2	0	0
12/m/47	2	+	416.0	1.16	26.2	4.19	1.37	<0.35	<0.35	3	1	3	0	ND
13/m/63	3	ND	159.0	0.69	1.91	2.90	1.68	<0.35	<0.35	3	2	3	0	0
14/f/23	1	+	177.0	<0.35	1.70	<0.35	<0.35	<0.35	<0.35	2	0	0	0	0
15/m/17	3	–	205.0	42.0	<0.35	3.70	<0.35	30.1	0.44	0	0	2	2	2
16/f/31	3	ND	197.0	0.42	<0.35	3.90	<0.35	<0.35	<0.35	1	3	1	0	0
17/m/42	2	ND	250.0	2.63	7.36	4.42	3.80	<0.35	<0.35	3	2	3	1	0

HMW, high-molecular-weight; AAI, alpha-amylase inhibitor; LTP, lipid transfer protein; ND, not done; f, female; m, male.

*The experimental semi-quantitative microarray immunoassay was performed using highly purified native wheat allergens (6). Purity was controlled by SDS-PAGE, acid PAGE, and/or MALDI-TOF. Individual cut-off levels for sIgE reactivity to each allergen component were estimated based on the fluorescence intensity for pools of negative samples and negative samples spiked with 3000 kU_A/l of myeloma IgE: 0 = negative (<400 fu); 1 = weakly positive (401–800 fu); 2 = positive (801–5000 fu); 3 = strongly positive (>5001 fu).

†Grading of anaphylactic reactions according to Ring and Messmer (10).

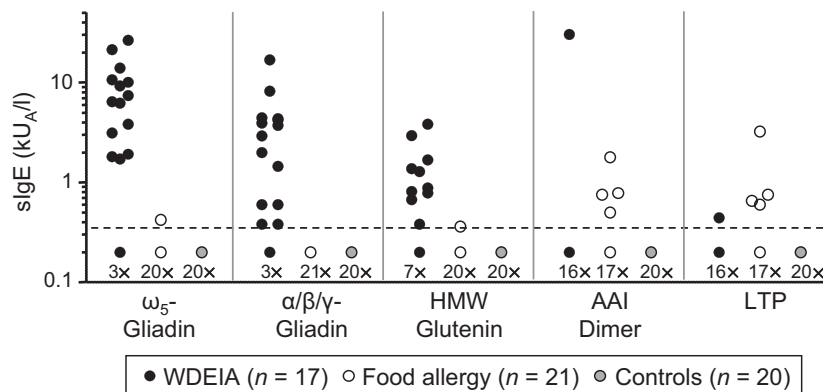


Figure 1 IgE reactivity to ω_5 -gliadin, α/β -gliadin, and γ -gliadin, high-molecular-weight (HMW) glutenin, alpha-amylase inhibitor (AAI), and wheat lipid transfer protein (LTP) in patients with wheat-dependent exercise-induced anaphylaxis (WDEIA) ($n = 17$), patients with

anaphylactic reaction to other food stuff ($n = 21$), and nonatopic controls ($n = 20$) as assessed by experimental ImmunoCAP assays. The number of sera with IgE levels below the cut-off (<0.35 kU_A/l) is listed for each parameter directly above the x-axis.

Contributions

SCH and JF acquired the data, performed data interpretation, wrote and revised the article. CE and OBG performed analysis,

performed data interpretation, wrote and revised the article. TB and TJ developed the concept and design of the study, performed data interpretation, and wrote and revised the article. All authors have approved the final version of the manuscript.

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