ORIGINAL ARTICLE

Clinical and atopic features of patients with wheat allergy dependent on augmentation factors (WALDA) presenting with urticaria: a monocentric study

Atopy in omega-5 gliadin allergy

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Summary

Background. Clinical and laboratory features of wheat allergy dependent on augmentation factor (WALDA) are scarcely characterised as compared to wheat anaphylaxis dependent on augmentation factor (WANDA). In this study we assessed the pattern of comorbid atopic and gastrointestinal diseases and the sensitisation profile in patients with WALDA presenting with intermittent urticaria. **Methods**. We retrospectively assessed all patients with WALDA-urticaria in a tertiary referral center, with a combined gastrointestinal- allergy unit. WALDA diagnosis was based upon recognised clinical/serological criteria, Clinical, atopic features, allergy tests and gastrointestinal comorbidities were compared to a cohort of O5G negative patients sensitised/allergic to wheat and one of patients allergic to shrimp. **Results**. Overall, we recruited 11 patients with WALDA presenting with intermittent urticaria (median age 44 years, IQR 29-58, F:M ratio 1.7:1). Atopy was a frequent finding among patients (8/11, 72.7%), with food allergy (6/11, 54.5%) followed by respiratory allergies (5/11, 45.5%). Shrimp sensitisation was present in 8/11 patients (72.7%); half of them were also clinically reactive to shrimp. Irritable bowel syndrome (IBS) was present in 4/11 patients (36.3%). The prevalence of shrimp sensitization was 15.3%, (p=0.01), in a group (n=13) of O5G negative patients with wheat sensitisation/allergy (median age 31 years, IQR 27.7-52.0, F:M ratio 0.4:1), while IBS prevalence was 9% (p=0.12). In the group of patients with shrimp allergy (n=13) with or without allergic rhinitis, the prevalence of O5G positivity was 0% and that of IBS 7.7%. **Conclusions.** Patients with WALDA-urticaria seems to present specific demographic features (female sex) and atopic (shrimp sensitisation/allergy).

Keywords

Food; gut; shrimp; urticaria; wheat.

1. Introduction

IgE-mediated wheat allergy imposes a significant burden on patients due to potentially severe manifestations, including anaphylaxis, often characterised by cardiovascular involvement, as well as challenges related to management-, such as strict wheat avoidance and its associated nutritional impact (1-3).

Omega-5 gliadin (O5G) is the most common allergen involved in wheat anaphylaxis dependent on augmentation factors (WANDA), (1-4). In this form of IgE-mediated food allergy, allergic reactions usually occur when wheat ingestion is combined with a cofactor, such as physical exercise, nonsteroidal anti-inflammatory drugs, or alcohol. These reactions are usually severe, with frequent cardiovascular involvement, including cases of refractory anaphylaxis (1,5-7). However, more recently, cases with isolated urticaria/angioedema have been described (8-9). Consequently, the broader term wheat allergy dependent on augmentation factor (WALDA) has been coined, to encompass the wider spectrum of clinical manifestations beyond anaphylaxis and, including skin-limited presentationa (4). O5G is also the most frequent allergen in WALDA (11). Factors responsible for different symptom severity in wheat allergy are still elusive. Indeed, most clinical studies have focused on WANDA, identifying its main features, such as male sex and nonatopic background (1,12,14). In contrast, WALDA remains less characterized, with limited understanding of its etiopathogenesis, and no widely accepted diagnostic criteria. More importantly, male sex ,the defining features of WANDA, may not to be a distinguishing feature of WALDA, as suggested by the findings of an Italian study (9).

In this study, we investigated the clinical background and sensitisation profile of O5G positive patients with WALDA-urticaria to look for demographic aspects, comorbidities, clinical presentation patterns and laboratory markers, which that may serve as salient clinical features or potential biomarkers of this subset of wheat allergic patients. More specifically, we hypothesised that this skin-limited form of wheat allergy may involve specific demographic and clinical-immunological features.

2. Materials and methods

2.1 Patient population and study design

This was a monocentric, observational, retrospective study conducted in a tertiary referral centre specialized in the diagnosis and management of wheat-related disorders, including coeliac disease and wheat allergy, with a combined gastroenterology and allergy outpatient clinic (Fondazione IRCCS San Matteo, Pavia).

We herein retrospectively enrolled all consecutive adult (≥18 years) patients who were diagnosed with O5G allergy over the last five years (2021-2025), since the start of the allergy outpatient clinic.

The diagnosis of WALDA related to O5G was made through adaptation of the criteria proposed by Jiang et al (12) for wheat-dependent, exercise-induced anaphylaxis. More precisely, the diagnosis was based on the fulfilment of criteria 1,2,3 and 4 or 5. Criterion 1, which originally required the involvement of two or more systems, was adapted for the classification of patients with WALDA-urticaria, allowing skin-only manifestations to be sufficient. Recurrent urticaria was defined if >1 episode of acute urticaria occurred over 6 months, and it was not present daily and continuously for >6 weeks and it was not induced by physical factors. Patients with a concomitant history of episodes of physically induced urticaria or dermographism were also excluded.

With reference to criterium 3, skin prick tests were performed with a commercial extract for wheat (Lofarma, Italy) or with gluten flour (Spain) dissolved in saline solution 0.9%, specific IgE were determined for wheat (f4) omega-5 gliadin (f416), gluten (f79), gliadin mix (f98) by FEIA, (Immunocap, Thermofischer, Sweden).

Some patients of the present cohort were included in a previous work (9). Demographic (sex, age) and clinical data of patients were extracted and pseudo-anonymised from the electronic hospital records onto a pre-defined spreadsheet. Clinical data included clinical manifestations, atopic comorbidities, sensitization profile, general and allergy laboratory data, pharmacologic therapies and diets. In all cases, a diagnosis of coeliac disease was ruled out by means of serology (*i.e.*, anti-tissue transglutaminase antibodies IgA). *A posteriori*, clinical and atopic features of O5G positive patients were compared to control groups from the same centre, including all wheat-allergic/sensitised patients, who were O5G-negative, and a group of consecutive shrimp-allergic patients. All data that were not present in the electronic records or in the physicians' assessment forms were retrieved through a phone call with the patient. Informed consent was obtained from all patients. The study was performed as a clinical audit using routine collected clinical and laboratory data. All participants provided

written informed consent for the use of their data in an aggregated and anonymous format. The study was approved by the local ethics committee (0023744/22). All results are reported according to the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) recommendations for quality assurance. Due to privacy compliance, the raw data cannot be made public but can be shared by the corresponding author upon reasonable request.

2.2 Statistical analysis

Continuous data were described with the median and interquartile range (IQR; i.e., 25th-75th percentiles), and categorical data as counts and percent. Comparisons between two groups were performed using the Student t test for continuous variables and the Chi-Square test for categorical variables. Missing data were excluded from percentage calculation, when specified. Given the limited sample size and the exploratory purpose, no corrections for multiple testing were applied.

The software GraphPad Prism (Boston, USA) was used for all computations. A 2-sided p-value<0.05 was considered statistically significant.

3.Results

3.1 Demographic, clinical, and laboratory data of patients with WALDA

Overall, we recruited 11 patients (median age 44 years, IQR 29-58, F:M ratio 1.7:1). Demographic and clinical features are summarised in Table 1. All patients presented at diagnosis with a history of episodes of intermittent urticaria, with a median number of 7 episodes (IQR 4-11).

In seven patients (63.6%) the diagnosis was confirmed with the remission of clinical manifestations after wheat avoidance, while in four patients (36.3%) the diagnosis was based on a positive oral food challenge, all of whom developed urticaria during the procedure. More precisely, an open food challenge with wheat (100 g of boiled gluten-containing pasta) followed by physical exercise (10-15 minutes running, adjusted to individual fitness levels) (13) was offered to 10 patients, excluding patient presenting with anaphylaxis. Of these, only four accepted and underwent the challenge. Most patients deemed it unnecessary, due to the disappearance of urticaria with wheat avoidance and hence refused the food challenge. At least a three-month period of wheat avoidance was used to confirm the diagnosis. Additionally, anaphylactic reactions were documented in three

patients, further supporting the diagnosis. More precisely, in one patient who received the indication of avoidance of cofactors anaphylaxis occurred after gluten and in two patients who were not adherent to a gluten-free diet (Table 1).

Given the predominantly skin-limited manifestations, several competing differential diagnoses had to be considered and excluded, including chronic spontaneous urticaria, physical urticaria, *Helicobacter pylori* infection, coeliac disease, and nonsteroidal antinflammatory drug hypersensitivity, among others.

The median diagnostic delay, as calculated from the onset of symptoms (mostly urticaria) and diagnosis was 3 years, IQR 1-6.

Results of specific IgE and skin prick tests of the patients are reported in Table 2 and Table 3. As per protocol, all patients displayed IgE positivity to the allergen O5G, Table 2. The most frequently positive wheat allergenic fraction was gluten (n=6, out of nine tested patients), followed by gliadin (n=3 out of seven tested patients), as detected by specific IgE and prick tests with gluten flour and specific IgE, respectively.

Overall, most patients displayed higher levels of O5G IgE compared to IgE other wheat allergenic fractions, accounting for missing data, Supplementary Table.

Commercial skin prick tests were positive for wheat in only three patients (not performed in one patient), while skin prick tests with gluten flour were positive in four patients (not performed in three patients). IgE for wheat were positive in four patients (not performed in three patients, IgE for gluten were positive in six patients (not performed in two patients), while IgE for gliadin in three (not performed in four patients). Generally, gluten sensitisation detected by either prick test or IgE testing was more frequently positive than that to wheat.

To explore possible diagnostic or clinical markers of WALDA or its clinical features, correlation analyses were performed. No statistically significant correlation was found between diagnostic delay and O5G specific IgE ($p=0.2$), total IgE levels ($p=0.7$) and age ($p=0.5$) and between O5G specific IgE and age ($p=0.4$), total IgE ($p=0.1$) and number of urticaria episodes ($p=0.3$)

3.2 Atopic and gastrointestinal diseases of patients with WALDA

Atopy was a frequent finding among patients (8/11, 72.7%), with food allergy (6/11, 54.5%) being more frequent than the respiratory allergies (5/11, 45.5%). No patient had eczema, drug or *Hymenoptera* venom allergy, among atopic comorbidities.

With regards to gastrointestinal comorbidities, irritable bowel syndrome (IBS) was a frequent finding in these patients being present in (4/11, 36.3%), according to the Rome IV criteria, while no cases of coeliac disease, inflammatory bowel disease, atrophic autoimmune gastritis, lactose intolerance were detected.

3.3 Sensitisation profile of patients with WALDA

Six patients (54.5%) were sensitised to house dust mite, three (37.3%) to Grass and two (18.1%) to Beach among respiratory allergens, Table 2 and 3, as assessed by specific IgE and/or skin prick tests, respectively. No patient was sensitised to weeds, animal dander, or moulds, Table 3. An extensive panel of commercial prick test for food allergens was negative except for shrimp (see later),Table 3.

Considering molecular allergens, three patients displayed positivity to the peach LTP, *Pru p 3*, two patients to PR-10, Bet v1, and one to *Phl p12, Table 2*. Among the patients sensitised to *Pru p 3*, only one was also positive for *Tri a 14,* but was unreactive to wheat when ingested at rest, hence reducing the likelihood that this allergen had a role in the clinical reactivity to wheat in addition to O5G.

3.4. Shrimp sensitization, related clinical symptoms and gastrointestinal comorbidities in patients with WALDA compared to control groups

Shrimp sensitization was a frequent finding, since eight out of the eleven patients (72.7%) were sensitized to shrimp, as assessed by either positive specific IgE (n=6) or skin prick tests (n=5). Three patients tested positive on both diagnostic modalities. Specific IgE to shrimp tropomyosin, *rPen a 1,* was detected in only two patients, Table 2. These patients were also sensitized to the house dust mite tropomyosin, *Der p 10*. In these patients,the higher levels of *Der p 10* compared to *Pen a 1,* with a 2:1 ratio, suggest a primary respiratory route of sensitization to this allergen. Indeed, one of these patients also reported perennial rhinitis related to dust mite exposure.

Among shrimp-sensitised patients (n=8), four (36.3% of the whole cohort), exhibited clinically reactive to shrimp. More precisely, three cases presented urticaria and one anaphylaxis. Notably, these reactions occurred after consuming shrimp without wheat co-ingestion. Moreover, in three of these four patients allergic reactions to shrimp temporally preceded those to wheat, Table 4, suggesting that shrimp allergy occurred before wheat allergy in this patient subset.

Correlation analysis revealed no statistically significant association between O5G IgE and shrimp and *D. pteronyssinus* specific IgE (Spearman's rho 0.23, p =0.2 and Spearman's rho 0.21, p=0.2, respectively).

We then compared the prevalence of shrimp sensitisation in patients with omega-5 allergy with that of a control group from our centre, consisting of patients sensitised/allergic to wheat, (n=13, median age 31 years, IQR 27.7-52.0, F:M ratio 0.4:1). These patients, whose characteristics are reported in Supplementary Table 1, were, by definition, O5G were more frequently male as compared to O5G positive ones (p=0.80). Sensitisation gluten (n=9) or the wheat LTP, *Tri a 14* (n=5, one patient was co-sensitised to both glutenin and *Tri a 14*) was observed in this group.

In this control group, the prevalence of shrimp sensitisation, as assessed by either positive specific IgE (n=2) or skin prick tests (n=1), with one patient being positive for both skin prick test and specific IgE, was observed in only 2 of 13 patients (15.3%). This prevalence was approximately four times lower compared to that of patients with O5G allergy (p= 0.01).

The prevalence of IBS was also assessed in this cohort and estimated at 9% (one in 11 patients, p=0.12 for comparison with patients with O5G allergy).

Finally, we assessed the prevalence of O5G sensitisation in a separate and unselected cohort (of patients with shrimp allergy n=13) with or without allergic rhinitis, Supplementary Table 2. None of these patient displayed IgE to O5G, nor positivity to wheat, gluten or gliadin. Eleven out of 13 (84%) patients were sensitised to house dust mite. The prevalence of IBS in this group was 7.7% (1/13), (p=0.08 for comparison with the prevalence in patients with O5G allergy).

Discussion and conclusions

In this retrospective real-life study, we assessed the prevalence of atopic and gastrointestinal comorbidities, as well as the sensitisation profile of patients with WALDA presenting with urticaria. The diagnosis was based on the adaptation of the criteria by Jiang (12) and already used in a preliminary communication by our group (9).

The first result of our study is the substantial prevalence of female sex and that of allergic comorbidities (atopy overall in 72.7% of patients) in those with WALDA presenting with urticaria. These features are in stark contrast with data from the European Anaphylaxis Registry from central Europe, since according to this study

male sex was more frequent, the prevalence of atopic comorbidities was collectively low and estimated at 36.9%, and cardiovascular symptoms were a very common finding (86.7%) in patients with wheat allergy (1). However, it is likely that, due to selection bias, mostly severe forms were enrolled in this registry, so that its results are probably not representative of a general outpatient population of wheat allergic patients. Of note, in a series from China, including patients with anaphylaxis, but also isolated urticaria, male sex was only slightly more common overall than female sex (12). It cannot be ruled out that the incorporation of patients with skin manifestations could have altered the male to female sex in this study, although information about sex in the subgroup of patients presenting with urticaria was not reported. Moreover, another interesting finding of this study is that in 64% of patients with anaphylaxis a history of previous recurrent urticaria was present. Hence urticaria may be a prodromal manifestation of more severe forms, at least in some patients. Alternatively, the presence of less severe skin manifestation may be related to the general lower level of physical exercise as a cofactor in female patients who are more represented in our studies of patients with WALDA presenting with isolated urticaria, as compared to WANDA (usually men) (2). An in-depth analysis of cofactors in our study was not performed. Yet, it also possible that the higher prevalence of female sex in patients with WALDA-urticaria underlies a biological phenomenon. Another result of our study is that the diagnostic delay of this form of allergy is significant (median 3 years, IQR 1-6), as compared to those series where anaphylaxis was reported among clinical manifestations (2,5). Some not exclusive explanations can be put forward. It is likely that these patients due to the intermittent nature of urticaria do not recognise wheat as the implied allergen, together with cofactors, and/or that, due the presence of isolated skin manifestations, they are not promptly referred to the allergist, while other specialists could be prioritised, such as the dermatologists. Another result of the study is that IBS was frequent in patients with WALDA, being present in 36.6% of patients. Interestingly, we did not find an association with other gastrointestinal diseases, including inflammatory bowel disease and coeliac disease, which are characterised by allergic comorbidities (14). While it is possible that this association could simply mirror the higher prevalence of IBS compared to the other gastrointestinal diseases, it is noteworthy that this finding was not replicated in patients with wheat allergy who were negative for O5G and that prevalence of IBS in omega-5 allergic patients was disproportionally high as compared to the general population (up to 11%) (15). Moreover, this finding lends itself to a mechanistic interpretation. Patients with IBS may self-impose wheat free diets to alleviate gastrointestinal

symptoms. Therefore, oral tolerance to wheat would be impaired, hence leading to the occurrence of wheat allergy. Unfortunately, we could not estimate the precise temporal sequence of development of IBS and wheat allergy in every patient to corroborate this hypothesis.

Another finding is the frequent rate of sensitisation of patients with WALDA to shrimp, in up to 72.7% of patients, with 36.3% of the whole cohort being also clinical reactive. This datum seems specific of patients with WALDA, since in the control group of wheat allergic patients not sensitised to O5G, the prevalence of shrimp sensitisation was four times lower (15%). On the contrary, no cases of omega-5 positivity were detected in patients sensitized to shrimp.

A considerable proportion of patients in our cohort with omega-5 had perennial rhinitis (5/11, 45.5%) and house dust mite sensitisation (6/11, 54.5%). Yet, given higher rate of sensitisation to shrimp (72.7%) compared to that of house dust mite, cross-reactivity between these allergenic sources cannot explain the sensitisation pattern in all patients. However, in the small subset of patients (n=2) with positive mice and shrimp tropomyosin, higher levels of IgE to *Der p 10*, the mite tropomyosin, than that of *rPen a 1*, the shrimp tropomyosin were detected. Hence, even though in only few patients both determinations were available, a primary respiratory route of sensitisation to this allergen is plausible. Moreover, half of the patients sensitised to shrimp were also clinically reactive and in these patients the reactivity to shrimp preceded that to wheat. Conversely, it has been hypothesised that shrimp sensitisation in patients with omega-5 allergy could be related to the high gluten diet used for crustacean farming (16). Since it is not known whether gluten is fully digested in this species, it is possible that undigested fragments may interact with shrimp proteins and hence lead to new allergens and/or promote shrimp sensitisation (12, 17). Yet this hypothesis needs formal validation. The mechanistic interpretation of the association between omega-5 sensitisation and that to shrimp is still elusive. The absence of a significant correlation between specific IgE levels for O5G and that of shrimp makes cross-reactivity between these two allergens a less likely explanation. ImmunoCap inhibition experiments could not be performed in the present study; however, a but they are envisaged as a future aspect of research to help clarify the eventual presence of a primary sensitiser. Nonetheless, screening patients with omega-5 allergy for shrimp allergy appears worthwhile, given the significant rate of clinical reactivity. Moreover, the evidence of shrimp sensitisation may suggest the diagnosis of WALDA in patients presenting with recurrent urticaria and

hence serve as a possible diagnostic biomarker. Indeed, in the correlation analysis no other demographic or laboratory marker, among those analysed, had an influence on the diagnostic delay.

The study has some limitations including the small sample of patients, the low rate of open food challenges, the possible selection bias of patients from a gastrointestinal unit and the main skin manifestation of WALDA in our cohort. Moreover, the enrolment of patients from a previous series may have introduced a bias. Larger, multicentre and prospective studies enrolling patients from other geographical regions and including non-cutaneous manifestations are needed in order to confirm these findings. While our focus was on O5G positivity, it is important to note that other wheat allergens also play a role in WALDA. For example, wheat lipid transfer protein (LTP), *Tri a 14*, is particularly relevant in the Mediterranean region (13). Sensitisation to O5G and *Tri a 14* appears to occur independent. Therefore, our findings may not be extendable to other WALDA populations defined by different serological profiles.

Collectively, the results of the present study suggest that patients with WALDA - urticaria may constitute a specific subset of wheat allergic patients and possibly a different phenotype, characterised by specific demographic features (female sex) and a high prevalence of atopic comorbidities, as opposed to patients with WANDA. Moreover, IBS is one of most frequent clinical nonatopic comorbidities of patients with WALDA-urticaria, implying that gastroenterological evaluation/referral in these patients is required. Finally, shrimp and house dust mite sensitisation/reactivity appears to be one relevant aspect, among others, in the management of allergic comorbidities in these patients.

Compliance with ethical standards

Funding

None.

Authors' contributions

CMR: study concept and design; CMR, SM, MDA: clinical management of patients; CMR, SM, MVL: analysis and interpretation of data, and manuscript preparation. SM statistical analysis and interpretation of data. ADS: critical revision for important intellectual contents, supervision. All the other authors interviewed and enrolled patients, locally collected data and reviewed the paper for final approval. All the authors provided approval of the final submitted version.

Conflict of interests

No conflict of interests to disclose**.**

Ethics approval and consent to participate

The study was approved by the local ethics committee.

Consent for publication

Informed consent from all patients was obtained.

Availability of data and materials

All data pertaining the study have been presented in the manuscript. Additional data can be asked to the corresponding author.

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Table 1. Demographic, clinical, and therapeutical features of O5G allergic patients

Abbreviations. A, alcohol; C, avoidance of gluten-containing foods in association with cofactors (physical exercise, anti-inflammatory drugs, alcohol, menstrual cycle, fever, ecc.); E, physical exercise; GFD, gluten-free diet; NSAID, non-steroidal anti-inflammatory Drug; OFC, oral food challenge¸ LTP, lipid transfer protein.

[?]Any food allergy in addition to wheat allergy.

Patient number	Sex	Age	Atopy	Eczema	Rhinitis	Asthma	Food allergy[?]	Food	1st food allergy	Diagnostic Delay (years)	Type Cofactor	OFC	Therapy at last follow-up	Anaphylaxis in the follow-up
1	M	58	Yes	No	Yes	No	No		Wheat	3	E	No	GFD	No
2	F	44	No	No	No	No	No		Wheat	5	E	No	GFD	Yes
3	M	29	No	No	No	No	No		Wheat	1	NSAID	Yes	GFD	No
4	M	52	No	No	No	No	No		Wheat	1	No	Yes	GFD	No
5	F	23	Yes	No	Yes	No	Yes	Peach	Wheat	6	E+ A	No	GFD	Yes
6	F	58	Yes	No	Yes	Yes	No		Wheat	13	E	No	C	No
7	F	46	Yes	No	No	No	Yes	Shrimp	Shrimp	2	E	Yes	C	No
8	F	22	Yes	No	No	No	Yes	Peach, shrimp	Wheat	1	E	No	GFD	Yes
9	F	32	Yes	No	No	No	Yes	Peach	Wheat	5	No	No	GFD	No
10	M	55	Yes	No	Yes	No	Yes	Shrimp	Shrimp	1	E	No	C	No
11	F	38	Yes	No	Yes	No	Yes	Shrimp	Shrimp	10	E+A	Yes	C	No

Table 2. Specific serum IgE levels of O5G allergic patients

Patient number	Total IgE	O5G	Wheat	Gluten	Gliadin	Shrimp	D. ptero	*Der p 1*	*Der p2*	*Der p 10*	*rPen a 1*	*Pru p 3*	*Tri a 14*	*Bet v 1*	*Phl p 12/ Bet v 2*
1	144	18.40	0.90	3.99	2.74	0.67	0.81	Neg	Neg	1.98	0.87	Neg	NA	13.5	Neg
2	375	4.49	0.25	1.75	0.61	0.14	Neg	NA	NA	NA	NA	Neg	NA	Neg	Neg
3	389	2.76	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	NA	Neg	0.32
4	147	1.72	Neg	0.47	0.21	1.46	Neg	Neg	Neg	Neg	Neg	Neg	NA	Neg	Neg
5	1147	7.04	3.59	1.69	NA	1.69	85.6	NA	NA	NA	NA	18.5	NA	9.51	Neg
6	1170	0.53	0.13	Neg	Neg	Neg	Neg	NA	NA	NA	NA	Neg	Neg	Neg	Neg
7	133	0.15	NA	NA	NA	0.15	0.11	NA	NA	Neg	Neg	Neg	Neg	Neg	Neg
8	115	0.23	NA	4.89	NA	NA	NA	NA	NA	NA	NA	0.36	Neg	Neg	Neg
9	534	0.93	NA	NA	NA	Neg	0.18	NA	NA	NA	NA	10.1	1.25	Neg	Neg
10	212	0.12	Neg	Neg	Neg	0.70	0.60	NA	NA	1.45	0.68	Neg	NA	Neg	Neg
11	18	0.52	Neg	0.15	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg

Abbreviations. *Bet v*, *Betula verrucosa;* D. ptero, *Dermatophagoides pteronyssinus*; *Der. p*, *Dermatophagoides pteronyssinus*; NA, not assessed; neg., negative if <0.10 kU/L; 05G, omega 5 gliadin, *rPen a*, recombinant *Penaeus aztecus*; *Phl p*, *Phleum pretense; Pru p*, *Prunus persica; Tri a*, *Triticum aestivum.*

Table 3. Skin prick tests of O5G allergic patients

Patient number	Wheat	Gluten*	HDM	Shrimp	Peach	Grass	Beach	Weed	Cat	Dog	Aspergillus	Alternaria
1	+	NA	-	+	-	+	+	-	-	-	-	-
2	+	NA	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-
4	-	+	-	+	-	-	-	-	-	-	-	-
5	NA	+	NA	NA	NA	NA	NA	NA	-	-	-	-
6	-	-	+	-	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-	-	-	-	-
8	-	+	-	+	+	+	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-	-	-	-	-
10	+	NA	+	+	-	-	-	-	-	-	-	-
11	-	+	-	+^	-	-	-	-	-	-	-	-

Abbreviations. HDM, house dust mite; NA, not available. In patient #5 skin prick tests were not performed due to histamine inhibition.

+denotes positive results; - denotes negative results. * denotes gluten flour. ^denotes prick by prick test.

Table 4. Clinical symptoms of patients with O5G allergy and shrimp sensitisation.

Patient number	Shrimp sensitisation*	Shrimp allergy	Shrimp allergy symptoms	First Symptomatic food	Perennial rhinitis	HDM sensitization*
1	Yes	No		Wheat	Yes	Yes
2	Yes	No		Wheat	No	No
3	No	No		Wheat	No	No
4	Yes	No		Wheat	No	No
5	Yes	No		Wheat	Yes	Yes
6	No	No		Wheat	Yes	Yes
7	Yes	Yes	Urticaria	Shrimp	No	Yes
8	Yes	Yes	Urticaria	Wheat	No	No
9	No	No		Wheat	No	Yes
10	Yes	Yes	Urticaria	Shrimp	Yes	Yes
11	Yes	Yes	Urticaria+rhinitis	Shrimp	Yes	No

Abbreviations. HDM, house dust mite. *Shrimp and HDM sensitisation was considered positive when either prick tests including prick by prick with fresh shrimp or serum IgE for shrimp were positive (>0.10 kU/L). Shrimp allergy diagnosis was made upon the combination of evidence of sensitization and consistent clinical manifestations.

Supplementary Table 1. Characteristics of wheat sensitised/allergic patients negative for omega-5 gliadin

Patient number	Sex	Age	Atopy	Eczema	Rhinitis	Asthma	Food allergy	Food	HDM SPT	Wheat SPT	Shrimp SPT	Wheat IgE	Gluten IgE	Gliadin IgE	Shrimp IgE	*rPen a 1* IgE	*Tri a 14* IgE
1	F	56	Yes	No	Yes	Yes	No	Wheat	NA	+	NA	0.13	0.13	Neg	Neg	NA	Neg
2	M	27	Yes	No	Yes	No	Yes	Peach	-	+	-	0.70	Neg	Neg	Neg	NA	0.12
3	F	19	Yes	No	No	No	Yes	Peach	+	+	-	1.25	Neg	Neg	Neg	NA	0.64
4	M	21	Yes	No	Yes	No	Yes	Peach	+	+	-	2.97	0.35	Neg	Neg	NA	0.40
5	M	70	Yes	No	No	No	Yes	Nuts	-	-	-	0.59	0.21	Neg	Neg	NA	Neg
6	M	40	Yes	No	No	No	Yes	Shrimp	-	-	+	0.14	0.50	Neg	0.66	Neg	Neg
7	F	28	Yes	No	Yes	No	No		-	-	-	0.75	0.11	Neg	Neg	NA	Neg
8	M	35	Yes	No	Yes	No	Yes	Wheat	-	+	-	2.26	2.08	0.57	Neg	Neg	Neg
9	F	48	Yes	No	Yes	No	No		+	-	-	Neg	0.52	Neg	Neg	NA	Neg
10	M	30	Yes	No	No	No	Yes	Shrimp	+	-	-	3.38	4.89	0.23	0.18	Neg	Neg
11	M	60	Yes	No	No	No	Yes	Peach	-	-	-	Neg	Neg	Neg	Neg	NA	0.29
12	M	31	Yes	No	No	No	Yes	Wheat	-	+	-	12.9	0.94	Neg	Neg	Neg	Neg
13	M	31	Yes	No	Yes	Yes	Yes	Peach	-	+	-	2.5	Neg	Neg	Neg	Neg	0.29

Abbreviations. HDM, house dust mite including *Dermatophagoides pteronyssinus* or *Dermatophagoides farinae*; neg., negative if <0.10 kU/L; NA, not assessed; *rPen a*, recombinant *Penaeus aztecus;* SPT, skin prick test. Wheat allergy diagnosis was made upon the combination of evidence of sensitization and consistent clinical manifestations

Supplementary Table 2. Characteristics of shrimp allergic patients

Patient number	Sex	Age	Atopy	Eczema	Rhinitis	Asthma	Food allergy[?]	Food	HDM SPT	Wheat SPT	Shrimp SPT	Wheat IgE	Gluten IgE	Gliadin IgE	Shrimp IgE	*rPen a 1* IgE	*Tri a 19 IgE*
1	F	57	Yes	No	Yes	No	No		+	-	+	Neg	Neg	Neg	5.72	3.45	Neg
2	F	26	Yes	No	Yes	No	Yes	Sesame	+	-	+	Neg	Neg	Neg	0.37	Neg	Neg
3	F	31	Yes	No	Yes	No	No		+	-	+	Neg	Neg	Neg	Neg	Neg	Neg
4	F	29	Yes	No	Yes	No	No		+	-	+	Neg	Neg	Neg	5.56	1.35	Neg
5	F	32	Yes	No	No	No	No		-	-	+	Neg	Neg	Neg	0.17	Neg	Neg
6	F	55	Yes	No	Yes	No	No		+	-	+	Neg	Neg	Neg	0.19	0.12	Neg
7	M	51	Yes	No	Yes	No	No		+	-	+	Neg	Neg	Neg	0.42	Neg	Neg
8	M	18	Yes	No	Yes	No	No		+	-	+	Neg	Neg	Neg	11.6	3.70	Neg
9	M	30	Yes	Yes	Yes	No	Yes	Fish	+	-	+	Neg	Neg	Neg	0.59	Neg	Neg
10	M	64	Yes	No	Yes	No	No		+	-	+	Neg	Neg	Neg	0.20	Neg	Neg
11	M	48	Yes	No	Yes	No	No		+	-	+	Neg	Neg	Neg	19.3	18.0	Neg
12	F	48	Yes	No	Yes	No	No		-	-	+	Neg	Neg	Neg	1.47	Neg	Neg
13	M	30	Yes	No	Yes	No	No		+	-	+	Neg	Neg	Neg	2.36	Neg	Neg

Abbreviations. HDM, house dust mite including *Dermatophagoides pteronyssinus* or *Dermatophagoides farinae*; neg., negative if <0.10 kU/L; NA, not assessed; *rPen a*, recombinant *Penaeus aztecus;* SPT, skin prick test.

[?] Any food allergy in addition to shrimp allergy. Shrimp allergy diagnosis was made upon the combination of evidence of sensitization and consistent clinical manifestations

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Supplementary Table 3. Missing data with absolute numbers and percentages for Table 1 and 2

Variable	Total patients (N=11)	Missing data (n)	Missing data (%)
Wheat IgE	11	3	27.3
Gluten IgE	11	2	18.2%
Gliadin IgE	11	4	36.4%
Shrimp IgE	11	1	9.1%
D. *ptero* IgE	11	1	9.1%
Der p 1 IgE	11	6	54.5%
Der p 2 IgE	11	6	54.5%
Der p 10 IgE	11	6	54.5%
rPen a 1 IgE	11	6	54.5%
Pru p 3 IgE	11	1	9.1%
Tri a 14 IgE	11	3	27.3%
Bet v 1 IgE	11	0	0%
Phl p 12 / Bet v 2 IgE	11	0	0%
Wheat SPT	11	1	9.1%
Gluten SPT	11	3	27.3%
Shrimp SPT	11	1	9.1%
HDM SPT	11	1	9.1%
Other allergen SPT	11	0–3*	up to 27%

Abbreviations. IgE, immunoglobulin E; *ptero*., *pteronyssinus* ; SPT,skin prick tests.
*depending on allergen.