

## The clinical spectrum of omega-5-gliadin allergy

### Authors

Thanh-Thao (Adriana) Le – MBBS (Percentage contribution to the paper: 40%)

Dual trainee in Clinical Immunology and Allergy, and General and Acute Care Medicine

Clinical and laboratory immunology registrar

Royal Adelaide Hospital/SA Pathology (Human Immunology)

Mahmood Al Kindi – MBBS, FRCPA (Percentage contribution to the paper: 10%)

Immunology registrar

SA Pathology (Human Immunology)<sup>1</sup>

1. Present address: Flinders Medical Science and Technology, Immunology, Allergy and Arthritis Department

Ju Ann Tan – MBBS, MRCP, FRACP (Percentage contribution to the paper: 5%)

Immunology registrar

Royal Adelaide Hospital<sup>2</sup>

2. Present address: University of British Columbia and Arthritis Research Centre of Canada

Anthony Smith – MBBS FRACP FRCPA PhD (Percentage contribution to the paper: 5%)

Clinical Immunologist

Flinders Medical Centre

Robert Heddle – MBBS FRACP FRCPA PhD (Percentage contribution to the paper: 5%)

Chief Pathologist, SA Pathology

Head, Clinical Immunology Unit, Royal Adelaide Hospital

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/imj.13091

Frank Kette – MBBS FRACP FRCPA PhD (Percentage contribution to the paper: 10%)

Clinical Immunologist

Royal Adelaide Hospital

Pravin Hissaria – MBBS MD FRCPA FRACP (Percentage contribution to the paper: 10%)

Clinical Immunologist and Immunopathologist

Royal Adelaide Hospital

SA Pathology (Human Immunology)

William Smith – MBBS FRACP FRCPA PhD (Percentage contribution to the paper: 15%)

Clinical Immunologist

Royal Adelaide Hospital

#### **Address for correspondence**

Dr William Smith

Clinical Immunology Unit

Level 9, Royal Adelaide Hospital

North Tce, Adelaide

SA 5000

Ph: (08) 8222 2953

Fax: (08) 8222 0020

Email address: [william.smith@health.sa.gov.au](mailto:william.smith@health.sa.gov.au)

#### **Acknowledgements**

None

## Abstract

*Background:* IgE-mediated allergy to the wheat protein omega-5-gliadin (O5G) is associated with wheat dependent exercise-induced anaphylaxis (WDEIA), where exercise acts as a cofactor triggering anaphylaxis after wheat ingestion. The wider application of O5G specific IgE (sIgE) testing has revealed that the manifestations of O5G allergy extend beyond WDEIA.

*Aims:* This study documents clinical manifestations in a large series of patients with sIgE to O5G.

*Methods:* A retrospective clinical audit was performed on adult patients with a positive O5G sIgE ( $>0.35\text{kU/L}$ ) between 2007 and 2013, compared with a control group who had negative O5G sIgE. Clinical characteristics and skin prick test (SPT) results were examined.

*Results:* 67 patients were characterised, 26 of whom presented with food-dependent exercise-induced allergy, whilst others presented with exercise-induced symptoms without apparent food association (16/67), idiopathic anaphylaxis (10/67), food-induced allergic symptoms without exercise (10/67) or recurrent acute urticaria (5/67). Specific IgE to O5G had 91% sensitivity and 92% specificity for wheat-related allergic symptoms. SPT had sensitivity of 92% and specificity of 84%.

*Conclusion:* WDEIA is the most common manifestation of O5G allergy but patients may present with a variety of allergic manifestations, and wheat allergy is not always obvious on history. Non-exercise cofactors or a lack of cofactors were identified in many patients. A distinctive feature of this allergy is that despite regular wheat ingestion, allergic reactions to

wheat occur infrequently. Testing for sIgE to O5G should be considered in patients presenting with exercise-induced urticaria/anaphylaxis, idiopathic anaphylaxis and recurrent acute (but not chronic) urticaria.

## (i) Text

### Background

Wheat ingestion and inhalation is associated with a variety of immunologically driven hypersensitivity disorders including coeliac disease, classic immediate food allergy, baker's asthma and food-dependent exercise-induced anaphylaxis (FDEIA).<sup>1</sup> FDEIA is a distinct subset of food allergy, characterised by the induction of anaphylaxis during or immediately following exercise, occurring up to several hours following the ingestion of the culprit food.<sup>2</sup> In FDEIA the presence of specific IgE (sIgE) to the ingested food is a necessary prerequisite, but exercise is required as a cofactor to trigger the allergic reaction. Wheat is the most commonly reported food associated with FDEIA.<sup>3,4</sup>

The wheat protein omega-5-gliadin (O5G, Tri a 19, a fraction (3-6%) of wheat gluten<sup>5</sup> has been identified as the major allergenic protein in wheat-dependent exercise-induced anaphylaxis (WDEIA).<sup>6,7</sup> O5G is relatively insoluble and usually poorly absorbed after wheat ingestion; exercise has been shown to facilitate O5G absorption across the gastrointestinal mucosa.<sup>8</sup> Concomitant ingestion of alcohol or aspirin has also been demonstrated to increase plasma gliadin levels in patients with a history of WDEIA.<sup>8,9</sup> In patients with sIgE to O5G, both alcohol and aspirin have been reported to trigger anaphylaxis with wheat ingestion in the absence of exercise,<sup>10-12</sup> while non-aspirin NSAIDs, stress, infection, heat and menses have also been implicated as potential cofactors.<sup>13</sup> Wheat-dependent exercise-induced allergic reactions may be less severe with only cutaneous signs such as urticaria, erythema, and angioedema, or may include anaphylactic features such as bronchospasm, gastrointestinal involvement and hypotension.

The most commonly used in-vitro test for sIgE to allergens is the ImmunoCAP® commercial assay, which is available to test for IgE directed either towards total wheat protein or to purified O5G. Specific IgE to O5G is present in up to 84% of patients with WDEIA, with a

specificity of 96%.<sup>14,15</sup> Specific IgE to wheat protein is less sensitive and specific in the diagnosis of WDEIA. In cases of WDEIA where O5G has been excluded as the culprit allergen, other wheat allergens such as high-molecular-weight (HMW) glutenin and  $\alpha/\beta/\gamma$  gliadin may be responsible.<sup>16</sup> Since the introduction of the ImmunoCAP® assay for O5G sIgE, our experience in the South Australian (SA) adult population suggests that O5G allergy is reasonably common.

We have surveyed the clinical spectrum of presentation of O5G allergy in a large number of cases to examine the variability in clinical presentation of allergic disorders associated with O5G sIgE. A secondary aim was to determine the utility of serum sIgE antibodies against O5G in the diagnostic process, following clinical history and skin prick testing.

## Methods

### *Study population*

A retrospective audit was performed on adult patients with positive serology for O5G sIgE reported by SA Pathology between 2007 and 2013. SA Pathology is the only service in SA to offer the O5G sIgE assay. The majority of requests were referred from the Royal Adelaide Hospital and Flinders Medical Centre (the two tertiary level hospitals offering adult immunology/allergy specialty services in South Australia) and two private immunology/allergy specialist practices.

### *Inclusion criteria*

- a. Positive O5G sIgE, defined as  $\geq 0.35$  kU/L
- b. Patient medical records accessible through lab report numbers or hospital record numbers
- c. Assessment by a specialist clinical immunologist
- d. Sufficient information available from medical records to characterise the nature of the clinical presentation, including initial presentation pattern and diagnosis, final diagnosis after

testing, and where available information regarding subsequent events and response to treatment.

e. Exclusion of other causes of anaphylaxis

f. Age  $\geq 18$  years

#### *Comparator group*

A sequential cohort of patients with similar clinical presentations who had been tested for O5G sIgE with negative results (defined as  $<0.35\text{kU/L}$ ) fulfilling above criteria b, c, d and f were utilised in order to calculate the sensitivity and specificity of O5G sIgE compared to wheat sIgE and wheat extract skin prick testing in the diagnostic process.

#### *Study plan and design*

Medical records were obtained from the Royal Adelaide Hospital, Flinders Medical Centre and Immunology consultant private practices. Pre-defined clinical and laboratory data were extracted, tabulated and de-identified.

#### *Specific IgE*

All patients had sIgE antibodies O5G (f416) with or without sIgE antibodies to wheat (f4) and total IgE measured using the ImmunoCAP® (Phadia, Uppsala, Sweden).

#### *Skin prick tests*

Where skin prick testing (SPT) was performed, extracts of wheat (Alyostal 105) and/or wheat flour (Alyostal 106) were used (Stallergenes, France). A positive result was a wheal diameter  $\geq 3$  mm greater than the negative control.

#### *SDS-PAGE*

Electrophoresis of commercial SPT wheat extracts was carried out in a 10 % polyacrylamide precast according to manufacturer's instructions at 180 mA for 1 h. The resolved proteins were transferred onto a nitrocellulose membrane. The membrane was stained with 0.1% Coomassie Brilliant Blue.

### *Ethics*

Approval for this project was obtained from the Royal Adelaide Hospital Human Research Ethics Committee (HREC).

### *Statistics*

Statistic calculations were performed using GraphPad Prism (software version 5.04). Graph figures were created using Microsoft Excel (software version 14.0).

## **Results**

### *Characterisation of patients with O5G allergy*

Between 2007-2013 there were 819 requests to SA Pathology to perform specific IgE to O5G, with 98 positive results (O5G sIgE of  $\geq 0.35$  kU/L) in 83 unique patients. Inclusion criteria was fulfilled in 73 of these patients, 67 of whom were considered to have a final diagnosis of wheat-associated allergy following assessment with history, relevant investigations and, if available, response to treatment. A final diagnosis of WDEIA was reached in 53 of these patients, while seven had wheat-dependent other cofactor induced anaphylaxis, and seven had wheat-dependent anaphylaxis with no cofactor implicated with any episode. Of the six patients with positive O5G sIgE who were thought not to have wheat-associated allergic symptoms, four had a preferred alternative diagnosis made, while two had ongoing episodes despite wheat exclusion.

Subsequent to this study, a further 43 unique patients with a positive O5G sIgE result have been identified by our laboratory between 2013 and 2015 (2½ years), highlighting the fact

that O5G allergy is not rare and is likely to be increasingly detected as awareness of the disorder expands.

### *Demographics*

The age at presentation ranged from 20-84, with a median age of 44 years. Males accounted for 60% of cases (40/67). There was a history of atopy in 48% (32/67). In the comparator group, 28% (22/79) were male, median age of presentation was 38 years (range 18 to 76 years).

### *Initial presentation pattern (pre-test diagnosis)*

Of all patients with positive O5G sIgE, food-dependent exercise-induced allergy was the most common presentation pattern (26/67) (Table 1). Importantly, wheat was identified as the likely culprit food on the initial history in only half of these patients (13/26). The next most common presentations were exercise-induced allergic symptoms (not food associated), idiopathic anaphylaxis, and food-associated allergic symptoms without a consistent associated cofactor. A minority of patients presented with recurrent acute urticaria; however, no patients presenting with chronic urticaria (defined as urticaria on most days of the week for more than six weeks, n=8) or recurrent angioedema without urticaria (n=3) had a final diagnosis of O5G allergy.

Seven patients additionally reported background chronic generalised pruritus. In two of these patients, wheat avoidance resulted in resolution of pruritus, with recurrence on rechallenge.

Approximately half the patients (34/67) had symptoms for greater than one year prior to presentation to and diagnosis by an immunology specialist. The time from initial onset of symptoms to diagnosis ranged from within six months of first episode to fourteen years (median 2.6 years).

### *Symptoms and episode severity*

Thirty patients were able to provide an estimate of the time from ingestion to the development of allergic symptoms, with a median time of 30 minutes (range 5 minutes-3 hours).

The most common symptoms were urticaria, pruritis and angioedema (Figure 1). More than half of the patients (35/67) had signs or symptoms during one or more episodes consistent with hypotensive anaphylaxis (defined as loss of consciousness or systolic blood pressure <90mmHg), with dyspnoea and wheeze the next most common non-cutaneous manifestation.

Almost 60% of patients had at least one episode of grade 3 severity (Brown grading of anaphylaxis).<sup>17</sup> Most patients had episodes of varying severity on different occasions (Figure 2). There was no correlation between severity grading of anaphylaxis and O5G sIgE level (data not shown,  $r=-.07$ ,  $N=67$ ,  $p=0.59$ ).

### *Cofactors*

The majority of patients (60/67) reported a cofactor with at least one episode (Figure 3), while over one-third (25/67) identified more than one potential cofactor. However, 17 patients reported cofactors with some episodes but not others. In addition to the well-recognised cofactors of exercise, alcohol, aspirin and NSAIDs, heat (eg. hot bath, showering, weather) and acute stress (eg. public speaking, performance) were commonly reported. In seven cases only non-exercise cofactors were identified. In three cases more than one cofactor was temporally linked with the development of allergic symptoms, or the most severe episode reported was associated with multiple cofactors.

Regular low-dose aspirin use was present in 13 out of 67 patients. A history of temporal association between analgesic non-aspirin NSAID use and an allergic episode was reported in a further five patients, four with ibuprofen and one with diclofenac. All of these patients had a history of being able to tolerate the NSAID on other occasions.

Seven patients had no identifiable cofactors with any episodes, however all tolerated wheat on many other subsequent occasions without symptoms.

### *Management*

Patients received adrenaline on at least one occasion for an acute allergic episode in 37% (25/67) of cases. Epipen adrenaline auto-injectors were prescribed as part of the specialist management plan for 61% (41/67) of patients. Combination avoidance (avoid wheat only

when cofactors present) was recommended in 34 patients, 14 of whom had follow-up documented (letters or phone call records). Seven out of these patients had recurrent episodes. In one dramatic case, a patient who previously only had grade 1 severity episodes inadvertently exerted himself after eating wheat and suffered grade 3 hypotensive anaphylaxis requiring an adrenaline infusion. Complete wheat avoidance was recommended in 22 patients, with follow-up in 13 cases. Three out of these patients had further allergic episodes, but a diagnosis of wheat-related allergy was maintained due to suspicion of inadvertent ingestion.

#### *Performance of O5G sIgE testing and SPT*

Using a cut-off value of  $\geq 0.35$  kU/L, O5G sIgE had a 91% sensitivity and 92% specificity for wheat-dependent cofactor associated allergy. Specificity improved to 99% when a cut-off value of  $\geq 1.0$  kU/L was used instead, albeit with reduction in sensitivity to 85%. Wheat sIgE had poor diagnostic sensitivity and specificity (64% and 56% respectively). In the 52 of 67 O5G allergic patients who had wheat sIgE performed, 21 had negative wheat sIgE ( $< 0.35$  kU/L).

Two commonly available wheat extracts used for skin prick testing in Australia are wheat grain (Alyostal 105) and wheat flour (Alyostal 106). In our cohort of 67 patients, 56 and 43 patients respectively had skin testing to Alyostal 105 and Alyostal 106 wheat extract. Alyostal 106 offered superior sensitivity (94%) to Alyostal 105 (63%) without marked decrease in specificity (82% and 87% respectively) for the diagnosis of wheat-dependent cofactor associated allergy. In addition, the average wheal size for Alyostal 106 (5.3mm) was greater compared to Alyostal 105 (3.0mm). Concurrent testing to both extracts was performed in 41 patients; 38 (93%) had a positive SPT with Alyostal 106, but only 24 (59%) with 105. The difference in sensitivity was presumably accounted for by the higher O5G content in Alyostal 106 (SDS-PAGE showed greater intensity of bands in the omega-gliadin region; data not shown).

#### *Serum tryptase (baseline and reaction)*

Baseline serum tryptase was measured in 57 patients, and was  $\leq 13$  mcg/L in all cases (reference range  $< 12$  mcg/L); none had a diagnosis of mast cell activation disorder. There was no correlation between baseline tryptase and symptom severity ( $r = -0.15$ ,  $N=57$ ,  $p=0.27$ ). Six patients had both a baseline mast cell tryptase level and tryptase level taken during an episode of anaphylaxis. Three patients (all with hypotensive anaphylaxis) had marked rise in tryptase above the reference range (baseline 2, 4 and 6 to 25, 31 and 45 respectively); the remaining three had an elevation of 3-4 mcg/L, consistent with anaphylaxis whilst still remaining within the reference range.<sup>18</sup>

## Discussion

Systemic food allergy reactions occur when antigenic food proteins or peptides are absorbed via the gastrointestinal tract and interact with specific IgE bound to its receptor on the surface of mast cells. In WDEIA, absorption of O5G is facilitated by exercise, hence the allergic reaction is conditional on exercise following wheat ingestion. Other cofactors such as aspirin, NSAIDs and alcohol may also facilitate O5G absorption and trigger reactions.

Although WDEIA is the most common and well-recognised manifestation of O5G allergy, we found that the initial diagnosis of FDEIA was made in only about 40% of our patient cohort, with wheat identified as the culprit food on the basis of presenting history in only half of these patients, prior to testing. Other presentation patterns included exercise-induced anaphylaxis without apparent food dependence, idiopathic anaphylaxis without cofactor dependence, and recurrent acute urticaria, thus illustrating variable severity and cofactor dependence as well as a lack of recognition of wheat-dependence at the time of presentation. Following a positive SPT or O5G sIgE result, further history confirmed wheat and cofactor dependence in many but not all patients, whereas in a significant minority of cases, wheat allergy reactions had occurred in the absence of any known cofactors.

Aspirin is a potential cofactor for O5G allergic reactions with or without exercise.<sup>19</sup> Thirteen of our 67 patients were taking regular aspirin in low dosage. The role of aspirin has been confirmed by studies in which plasma gliadin levels were increased when wheat was ingested following aspirin at a dose of 500mg<sup>8</sup> or 100mg<sup>20</sup>. Provocation studies have confirmed anaphylaxis in patients with O5G allergy with wheat ingestion following aspirin 500mg,<sup>10,19</sup> whilst some patients required both cofactors (aspirin 500mg, wheat ingestion, then exercise)

to provoke a reaction.<sup>19</sup> A case report confirms that WDEIA can occur in patients on low-dose (100mg) aspirin therapy.<sup>21</sup>

We found that cofactor dependence was variable between different patients and in the same patient on different occasions, and in some patients cofactors were apparently absent. Wheat was often tolerated on the majority of occasions without reaction, whereas reactions occurred intermittently and inconsistently in relation to wheat ingestion, often for no apparent reason. This appears to be a distinguishing feature of O5G allergy in adults compared to other forms of classical Type 1 food allergy. It is not known whether some of the variability relates to the quantity of wheat O5G ingested.<sup>22</sup> In a previous study of patients with WDEIA, 25% reacted to ingestion of high-O5G gluten flour alone, in the absence of exercise.<sup>9</sup> Different wheat products are likely to contain varying levels of O5G protein and it is possible that different processing or cooking methods may alter O5G content and allergenicity. Some of our patients reported the ability to tolerate some forms of wheat products but not others, although there was no consistent pattern. It is not currently possible to recommend a particular wheat product to patients with O5G allergy, but it may be feasible to intentionally modify wheat products to have negligible O5G content.<sup>23,24</sup>

Wheat avoidance will eliminate the risk of anaphylaxis, but patients can be reluctant to undergo a wheat exclusion diet, especially when symptoms are variable and infrequent. Patients may therefore prefer to avoid the combination of wheat and cofactors. It is generally advised that food ingestion and cofactor exposure be separated by a minimum of four hours in most patients.<sup>3</sup> In our patients, 7/14 who undertook combination avoidance and 3/13 who managed with wheat avoidance experienced repeat episodes. It seems prudent for patients with more severe episodes to be advised to practice wheat elimination.

The potential cross-reactivity between allergens from rye, barley and oats has previously been studied in immunoblot inhibition and ELISA studies in patients with O5G-related WDEIA, with demonstration of inhibition and IgE-binding to rye  $\gamma$ -75k-secalin and barley  $\gamma$ 3-hordein.<sup>25</sup> However, clinical reactions to rye and barley in WDEIA patients have not been observed. Whilst it is not necessary to avoid non-wheat sources of gluten, in practice a gluten-free diet may be a convenient default. Since a high proportion of patients has at least one severe reaction, the severity of future reactions cannot be reliably predicted, and there is a significant risk of further reactions whether combination avoidance or wheat elimination is

the recommended strategy, specialist review and an EpiPen prescription is highly recommended for most if not all with O5G allergy.

In our patients O5G sIgE had a high sensitivity (91%) and specificity (92%) for wheat-related cofactor induced allergy with a cut-off value of  $\geq 0.35$  kU/L. With a cut-off value of  $\geq 1.0$  kU/L, the specificity of O5G sIgE increased to 99%. Critical evaluation of the diagnosis becomes more important at O5G sIgE levels between 0.35 and 1.0 kU/L. Matsuo et al found similar results with improved specificity at a cut-off value of 0.89 kU/L rather than 0.35 kU/L (96% vs 68% respectively) without loss of sensitivity (78% vs 80% respectively).<sup>15</sup> In contrast, wheat sIgE has a poor sensitivity and specificity in the diagnosis of wheat-related cofactor induced allergy (both O5G and non-O5G related). The level of specific IgE to O5G did not predict reaction severity in our patients.

Regarding skin prick testing, our results suggest different wheat extracts contain variable levels of O5G protein, greatly affecting test sensitivity. Therefore while SPT can be a useful diagnostic modality in the diagnosis of O5G allergy, it is dependent on the availability of a suitably sensitive extract.

The presence of sIgE to food does not necessarily indicate food allergy, and when the history is not clear, oral food challenge (OFC) is the gold standard for diagnosis. In the case of O5G this is difficult due to multiple factors including cofactor variability, unpredictable reaction severity and practical difficulties. SPT using an extract with high gliadin content is a very effective test in specialist practice. Specific IgE to O5G appears to offer excellent diagnostic performance for clinicians in diagnosing WDEIA and related forms of wheat allergy, and awareness of the variety of possible clinical manifestations will reduce the risk of missed or delayed diagnosis.

## Conclusion

WDEIA is the most common manifestation of O5G allergy but patients may present with a variety of allergic manifestations, and relationship to wheat and cofactor exposure is not always obvious on the history. Testing for specific IgE to O5G and/or SPT with a sufficiently sensitive wheat extract should be considered in patients presenting with exercise-induced urticaria/anaphylaxis, idiopathic anaphylaxis and recurrent acute urticaria if there is a feasible

connection to wheat ingestion. Patients with suspected O5G allergy should be referred to an allergy specialist for confirmation of diagnosis and management.

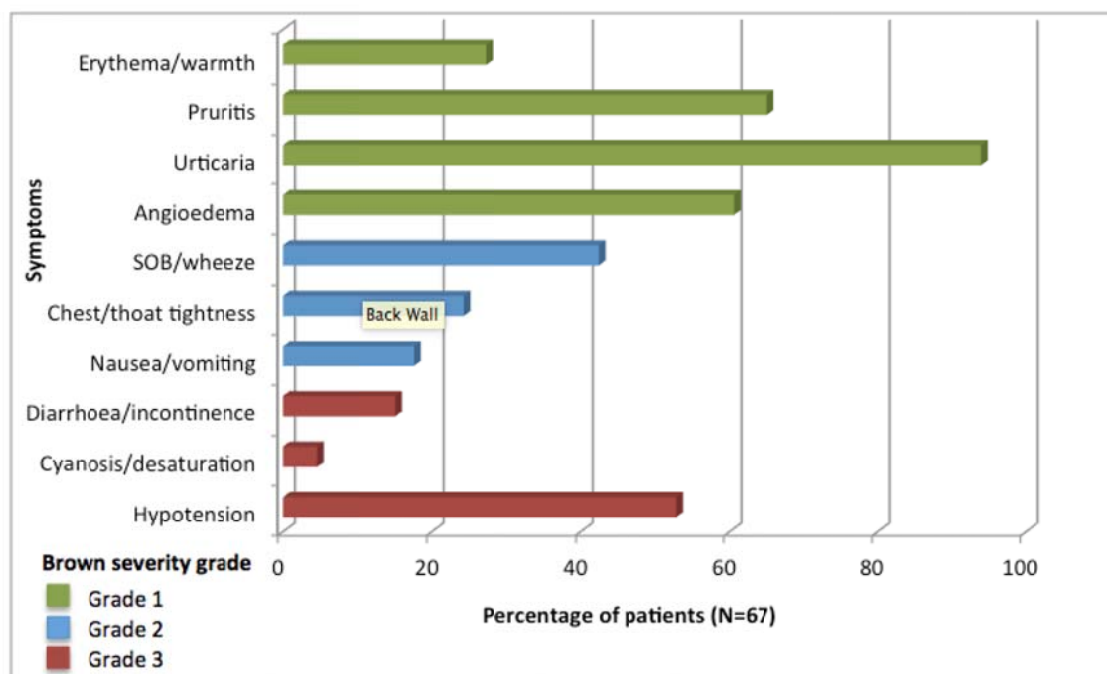
**(ii) References**

1. Palosuo K. Update on wheat hypersensitivity. *Curr Opin Allergy Clin Immunol* 2003; 3(3): 205-9.
2. Kidd JM, Cohen SH, Sosman AJ, Fink JN. Food-dependent exercise-induced anaphylaxis. *J Allergy Clin Immunol* 1983; 71: 407–11.
3. Morita E, Kunie K, Matsuo H. Food-dependent exercise-induced anaphylaxis. *J Dermatol Sci.* 2007 Aug;47(2):109-17.
4. Beaudouin E, Renaudin JM, Morisset M, Codreanu F, Kanny G, Moneret-Vautrin DA. Food-dependent exercise-induced anaphylaxis--update and current data. *Eur Ann Allergy Clin Immunol.* 2006 Feb;38(2):45-51.
5. Wieser H: Chemistry of gluten proteins. *Food Microbiol* 2007; 24: 115–119.
6. Palosuo K, Alenius H, Varjonen E et al. A novel wheat gliadin as a cause of exercise induced anaphylaxis. *J Allergy Clin Immunol* 1999; 103: 912–7.
7. Morita E, Matsuo H, Mihara S, Morimoto K, Savage AWJ, Tatham AS. Fast omega-gliadin is a major allergen in wheat-dependent exercise-induced anaphylaxis. *J Dermatol Sci* 2003; 33: 99–104.
8. Matsuo H, Morimoto K, Akaki T, Kaneko S, Kusatake K, Kuroda T, et al. Exercise and aspirin increase levels of circulating gliadin peptides in patients with wheat-dependent exercise-induced anaphylaxis. *Clin Exp Allergy.* 2005; 35: 461–6.
9. Brockow K, Kneissl D, Valentini L, Zelger O, Grosber M, Kugler C, Werich M, et al. Using a gluten oral food challenge protocol to improve diagnosis of wheat-dependent exercise-induced anaphylaxis. *J Allergy Clin Immunol.* 2015 Apr;135(4):977-84.e4.
10. Matsukura S, Aihara M, Sugawara M, Kunimi Y, Matsuki M, Inoue Y, et al. Two cases of wheat-dependent anaphylaxis induced by aspirin administration but not by exercise. *Clin Exp Dermatol* 2010;35:233-7.
11. Fiedler EM, Zuberbier T, Worm M. A combination of wheat flour, ethanol and food additives inducing FDEIA. *Allergy* 2002;57:1090-1.

12. Wong GK, Huissoon AP, Goddard S, Collins DM, Krishna MT. Wheat dependent exercise induced anaphylaxis: is this an appropriate terminology? *J Clin Pathol.* 2010 Sep;63(9):814-7.
13. Cardona V, Luengo O, Garriga T, Labrador-Horrillo M, Sala-Cunill A, Izquierdo A, Soto L, Guilarte M. Co-factor-enhanced food allergy. *Allergy* 2012; 67: 1316-18.
14. Matsuo H, Morita E, Tatham AS et al. Identification of the IgE-binding epitope in  $\alpha$ -5 gliadin, a major allergen in wheat-dependent exercise-induced anaphylaxis. *J Biol Chem* 2004; 279:12135–40.
15. Matsuo H, Dahlstrom J, Tanaka A, Kohno K, Takahashi H, Furumara M, Morita E. Sensitivity and specificity of recombinant  $\alpha$ -5 gliadin-specific IgE measurement for the diagnosis of wheat-dependent exercise-induced anaphylaxis. *Allergy* 2008; 63:233-36.
16. Hofmann SC, Fischer J, Eriksson C, Bentsson, Gref O, Biedermann T, Jakob T. IgE detection to  $\alpha/\beta/\gamma$ -gliadin and its clinical relevance in wheat-dependent exercise induced anaphylaxis. *Allergy* 2012; 67: 1457-60.
17. Brown S. Clinical features and severity grading of anaphylaxis. *J Allergy Clin Immunol.* 2004 Aug;114(2):371-6.
18. Brown S. Can serum mast cell tryptase help diagnose anaphylaxis? *Emerg Med Australas* 2004;16(2):120-4.
19. Harada S, Horikawa T, Ashida M, Kamo T, Nishioka E, Ichihashi M Aspirin enhances the induction of type I allergic symptoms when combined with food and exercise in patients with food-dependent exercise-induced anaphylaxis. *Br J Dermatol.* 2001 Aug;145(2):336-9.
20. Matsuo H, Kaneko S, Tsujino Y et al. Effects of nonsteroidal anti-inflammatory drugs (NSAIDs) on serum allergen levels after wheat ingestion. *J Dermatol Sci.* 2009 Mar;53(3):241-3.
21. Fujii H, Kambe N, Fujisawa A, Kohno K, Morita E, Miyachi Y. Food-dependent exercise-induced anaphylaxis induced by low dose aspirin therapy. *Allergol Int* 2008;57: 97-8.

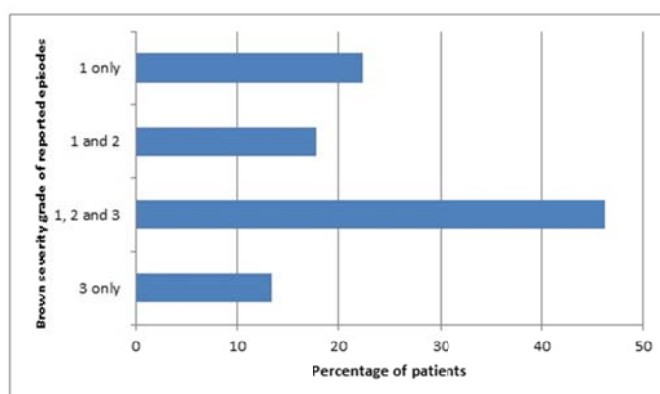
22. Scherf K, Brockow K, Biedermann T, Koehler P, Wieser H. Wheat-Dependent Exercise-Induced Anaphylaxis. *Clin Exp Allergy*. 2016 Jan;46(1):10-20.
23. Altenbach SB, Allen PV. Transformation of the US bread wheat 'Butte 86' and silencing of omega-5 gliadin genes. *GM Crops*. 2011 Jan-Mar;2(1):66-73.
24. Altenbach SB, Tanaka CK, Seabourn BW. Silencing of omega-5 gliadins in transgenic wheat eliminates a major source of environmental variability and improves dough mixing properties of flour. *BMC Plant Biol*. 2014 Dec 24;14:393.
25. Palosuo K, Alenius H, Varjonen E, Kalkkinen N, Reunala T. Rye gamma-70 and gamma-35 secalins and barley gamma-3 hordein cross-react with omega-5 gliadin, a major allergen in wheat-dependent, exercise-induced anaphylaxis. *Clin Exp Allergy*. 2001 Mar;31(3):466-73.

**(iii) Figure legends**

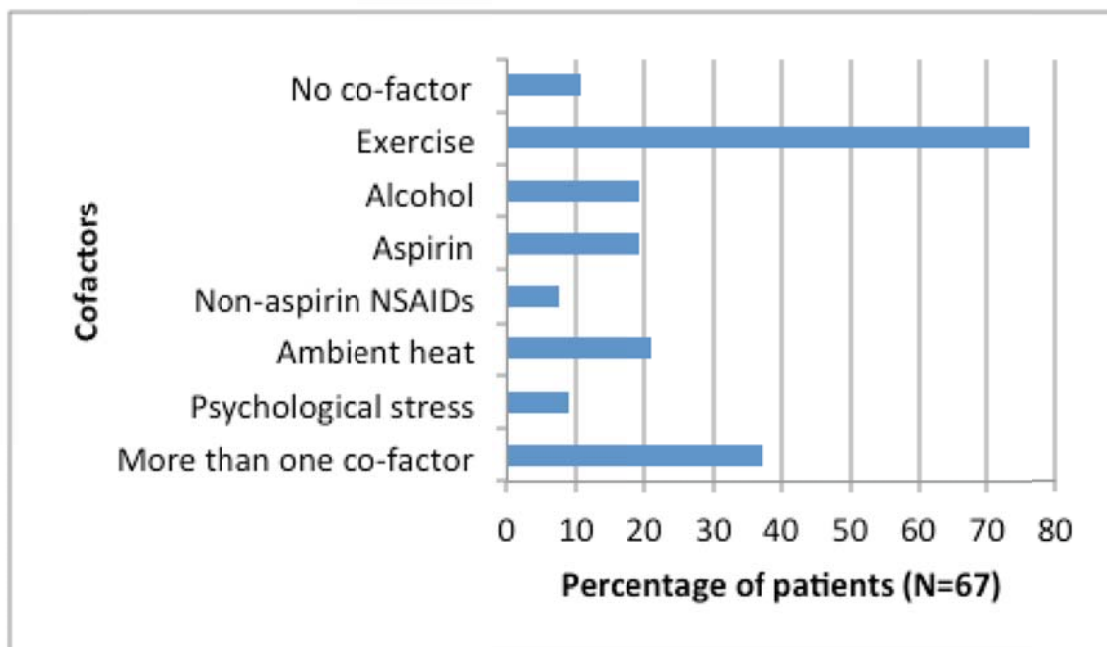


- Figure 1 – Frequency of symptoms and signs classified by Brown Anaphylaxis Grading in patients with O5G allergy.

- Figure 1 footnote – Brown grading system for severity of generalised hypersensitivity reactions: Grade 1 – Mild (skin and subcutaneous tissues only): generalised erythema, urticaria, periorbital oedema, or angioedema; Grade 2 – Moderate (features suggesting respiratory, cardiovascular, or gastrointestinal involvement): dyspnoea, stridor, wheeze, nausea, vomiting, dizziness(presyncope), diaphoresis, chest or throat tightness, or abdominal pain; Grade 3 – Severe (hypoxia, hypotension, or neurologic compromise): cyanosis or  $\text{SpO}_2 \leq 92\%$ , hypotension ( $\text{SBP} < 90 \text{ mm Hg}$ ), confusion, collapse, LOC, or incontinence.



- Figure 2 – Individual range in severity of allergic episodes in patients with O5G allergy.



- Figure 3 – Reported cofactors in patients with O5G allergy.

**(iv) Tables**Table 1: Initial presentation pattern in 67 patients with O5G allergy

| Presentation                                                        | No. of pts (N=67) |
|---------------------------------------------------------------------|-------------------|
| Wheat-dependent exercise-induced urticaria or anaphylaxis (WDEIU/A) | 13                |
| Food-dependent exercise-induced allergy (FDEIA)                     | 13                |
| Exercise-induced urticaria or anaphylaxis (EIU/A)                   | 16                |
| Idiopathic anaphylaxis (IA)                                         | 10                |
| Food-induced allergy (FIA)                                          | 10                |
| Recurrent acute urticaria (RAU)                                     | 5                 |