

ORIGINAL ARTICLE

Wheat IgE profiling and wheat IgE levels in bakers with allergic occupational phenotypes

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ABSTRACT

Objectives To characterise occupational wheat allergic phenotypes (rhino-conjunctivitis, asthma and dermatitis) and immunoglobulin (IgE) sensitisation to particular wheat allergens in bakers.

Methods We conducted clinical and immunological evaluations of 81 consecutive bakers reporting occupational symptoms using commercial tests (skin prick test (SPT), specific IgE, ISAC microarray) and six additional dot-blotted wheat allergens (Tri a 39, Tri a Trx, Tri a GST, Tri a 32, Tri a 12, Tri a DH).

Results Wheat SPT resulted positive in 29 bakers and was associated with work-related asthma ($p < 0.01$). Wheat IgE was detected in 51 workers and was associated with work-related asthma ($p < 0.01$) and rhino-conjunctivitis ($p < 0.05$). ISAC Tri a 30 was positive in three workers and was associated with work-related dermatitis ($p < 0.05$). Wheat dot-blotted allergens were positive in 22 bakers. Tri a 32 and Tri a GST were positive in 13 and three bakers, respectively, and both were associated with work-related dermatitis ($p < 0.05$). This association increased ($p < 0.01$) when Tri a 32, Tri a GST and Tri a 30 were analysed together ($p < 0.01$). Wheat IgE levels were associated with work-related dermatitis ($p < 0.01$).

Conclusions Wheat IgE levels and wheat microarrayed allergens may be associated with some occupational allergic phenotypes. The extension of the panel of wheat allergens may be promising for discriminating the clinical manifestations of baker's allergy.

INTRODUCTION

Previous studies have suggested a role for specific immunoglobulin (IgE) in work-related allergies among bakers,¹ although IgE positivity to flour explains the onset of rhinitis and/or respiratory symptoms in only 30%–40% of cases.² The use of skin prick test (SPT) based on wheat extracts has been fundamental to the diagnosis of baker's allergies and remains the most frequently used diagnostic test in this context.³ Wheat specific IgE detection, either by IgE-Enzyme Allergosorbent test or by Phadia CAP-system, is currently available as a diagnostic test with a higher sensitivity than SPT with commercial wheat extracts.⁴ However, the use of these 'in vivo' and 'in vitro' extract-based tests such as these is limited because such tests define the source of the exposure, rather than the allergenic molecules eliciting the disease.⁵ In recent years, microarray technology has been introduced and the

What this paper adds

- The role of allergenic molecules eliciting baker's allergy is not defined yet. Wheat skin prick test and wheat IgE positivity and levels may be associated with specific clinical phenotypes. The ISAC 103 array offers the possibility to analyse the role of wheat allergens and their association with clinical features.
- Our study shows that adding further wheat dot-blotted allergens to the ISAC panel supports the hypothesis that a larger panel of wheat allergens may be helpful to define the wheat allergic phenotype in bakers either for health surveillance purposes or during the patient's follow-up.

analysis of a large number of allergens in a single test has been made possible by measuring specific IgE antibodies against recombinants or purified natural allergen components, either in non-occupational or occupational setting.⁵

Sensitisation to latex, an important cause of occupational allergies, serves as a good example of how our diagnostic and epidemiological approaches have changed in recent years through the use of a panel of latex allergens. In a recent review,⁶ particular latex molecular allergens have been described to be major allergens in specific clinical phenotypes as in healthcare workers (Hev b 5 and Hev b 6), in multi-operated patients (Hev b 11) as those suffering from spina bifida and in patients with the so-called 'latex-fruit syndrome' (Hev b 6).

In wheat allergy, ω -5-gliadin (Tri a 19) is the first allergen associated with a clinical phenotype, namely, wheat-dependent exercise-induced anaphylaxis (WDEIA),⁷ and it has been included in the ISAC 103 microarray. In this panel, other wheat allergens such as agglutinin isolectin 1 (Tri a 18), gliadin fraction (Tri a Gliadin) and α -amylase/trypsin inhibitor (Tri a 30) are also included, but until now none of them has been clearly linked to any clinical phenotype. Recent efforts to characterise the molecular and immunological features of wheat allergens suggest their roles as possible markers for certain clinical manifestations in wheat allergy.^{8–10}

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Workplace

To our knowledge, there are no studies evaluating in bakers the correlation between clinical phenotypes and currently available diagnostic tests (wheat SPT, wheat IgE and ISAC array) and the correlation between allergic phenotypes and sensitisation to particular wheat ISAC microarray allergens. We conducted the present study to evaluate these correlations and the role of six additional wheat allergens previously described as having a role in baker's asthma.^{8–11} We also evaluated the association between occupational allergic phenotypes and wheat IgE levels.

MATERIAL AND METHODS

Study population

The baseline study population was identified through a cross-sectional postal survey of bakery workers in the Verona area. The survey was performed by the Occupational Medicine Unit of Verona University Hospital in association with the local Bakery Professional Association. The aim of the survey was to evaluate the prevalence of occupational allergic disorders and the relative risk factors.¹² All participants reporting suspected allergic symptoms at work (n=208) were invited to our centre to undergo a clinical evaluation; 81 (38.9%) accepted. Eight workers were bakery's counter-hands. Written informed consent to perform clinical and immunological tests was obtained from all patients and the local ethical committee approved the study.

Lung function tests

Each patient underwent baseline spirometry (SensorMedics V-Max 22) and, in the absence of airway obstruction, methacholine challenge (MB3 dosimeter, Mefar, Italy) according to guidelines.^{13–14} The methacholine challenge was stopped when a cumulative dose of 2 mg of methacholine was reached or when the forced expiratory value at 1 s (FEV₁) had fallen by 20% or more below the best baseline FEV₁ following diluent inhalation (PD₂₀ FEV₁). The test was considered as positive for a provocative dose of methacholine (PD₂₀ FEV₁) ≤1 mg. As they were not available in our facility, wheat challenge tests were not performed.

Skin prick tests

Commercial SPT extracts included pollen (birch, olive tree, grass, Parietaria, Artemisia, Ambrosia), cat dander, *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, *Alternaria alternata*, *Cladosporium herbarum* and *Blattella germanica*. Yeast, flours (wheat, rye, barley, oat and soy) (Lofarma, Milano, Italy) and α-amylase allergen (Stallergens, Antony, France) were considered as occupational allergens. Histamine phosphate (10 mg/ml) and normal saline were used as positive and negative controls, respectively. Positive SPT was defined as a weal diameter ≥3 mm. Atopy was considered to be present with at least one positive SPT to any of the environmental allergens included.

IgE determination

Total IgE and specific IgE to occupational allergens (yeast, α-amylase and flours (wheat, rye, barley, oat and soy)) were performed by ImmunoCAP (Phadia, Uppsala, Sweden). Specific IgE values of 0.35 kU/l or more were considered positive.

Clinical evaluation and diagnostic criteria

The clinical interview, performed by the same physician, included a standardised questionnaire based on the European Community Respiratory Health Survey¹⁵ concerning family or personal history for asthma, rhinitis, eczema, smoking status, and the onset of nasal and respiratory symptoms after exposure

to house dust, animals or pollen. Additional questions on respiratory (wheezing, whistling, chest tightness, shortness of breath), ocular (itchy or watery eyes), nasal (watery nose, sneezing, itching and nasal congestion) and skin symptoms (itchy skin, rash, and skin fissuring/crusts) at work were also assessed. Structured questions on occupational history, latency period between exposure and onset of symptoms, clinical characteristics of symptoms, medication assumption, use of respiratory protective equipment and the improvement of symptoms during days away from work were assessed as well. Subjects reporting more than one symptom at work were considered multi-symptomatic. Medical notes of other physicians documenting the patient's history of symptoms, once collected and recorded, were used to corroborate our clinical findings. Each patient underwent a clinical examination. None of the patients reported symptoms after eating wheat-derived products.

Bakers with skin fissuring and crust, skin erythema, and itchy skin at the clinical evaluation were diagnosed with work-related dermatitis. In the same way, bakers with ocular and nasal symptom at the clinical evaluation were diagnosed with work-related rhino-conjunctivitis. Bakers with shortness of breath and/or wheezing at work and with baseline airway obstruction or airway hyperresponsiveness at the methacholine challenge were diagnosed with work-related asthma.

Allergen microarray IgE detection

The ISAC microarray system (Phadia Multiplexing (PMD), Vienna, Austria) bearing 103 native or recombinant allergens from pollen, food, animal dander, mites, latex, mould and insects was performed. As occupational allergen, the ISAC system includes soy, sesame and four wheat allergen components: agglutinin isolectin 1 (nTri a 18), gliadin (nTri a Gliadin), ω-5-gliadin (rTri a 19.0101) and α-amylase/trypsin inhibitor (nTri a 30). IgE ISAC test has been performed as previously reported.¹⁶ At the end of each run, IgE data were exported to the InterAll software (Allergy Data Laboratories, Latina, Italy) using real-time connectivity¹⁶ and combined with demographic information, clinical data and results from IgE dot blotting (see below). Six recombinant wheat seed allergens,⁹ designated as #10 (Tri a 39), #37 (Tri a Trx), #38 (Tri a Glutathione-S-transferases (GST)), #112 (Tri a 32), #123 (Tri a 12) and #126 (Tri a Dehydrin (DH)), have been previously isolated from a cDNA library by screening with sera from baker's asthma patients and expressed in *Escherichia coli*. The above allergens have been tested by the dot-blot technique, as previously described.^{8–17} Detailed information about tested wheat allergens is shown in table 1.

Statistical analysis

Descriptive statistics are presented as mean±SD. The significance of differences between proportions was calculated using contingency tables and the χ^2 test or the Fisher's exact test, as appropriate. Linear regression models were used to assess the association of quantitative variables with the trait of interest. Quantitative variables not showing a normal distribution were log transformed to approximate to a normal distribution. Fisher's exact test was used to determine the statistical significance when comparing workers positive to immunological tests and clinical phenotypes. The value of association was estimated by calculating ORs with 95% CIs. Linear regression was performed to estimate the β-coefficient of the relationship between wheat IgE levels and the number of the symptoms at work presented by each individual. p Values less than 0.05 and greater or equal to 0.01 are reported as p<0.05 and p values less than

Table 1 Allergen nomenclature and cross-references

Allergen*	Clone name	Protein family	Nature	Testing	IUIS†	GeneBank‡	Uniprot‡
Tri a 12	#123	Profilin	Recombinant	Dot blot	NA	EU584499	D0PRB5
Tri a 12.0101					Tri a 12.0101		P49232
Tri a 12.0102					Tri a 12.0102		P49233
Tri a 12.0103					Tri a 12.0103		P49234
Tri a 12.0104					Tri a 12.0104		B6EF35
Tri a 18		Agglutinin	Natural	ISAC 103	Tri a 18		P10968
Tri a 19.0101		ω -5-Gliadin	Recombinant	ISAC 103	Tri a 19.0101	AB181300	Q402I5
Tri a 30		α -Amylase/trypsin inhibitor	Natural	ISAC 103	Tri a 30		P17314
Tri a 32	#112	1-Cys peroxiredoxin	Recombinant	Dot blot	NA	EU584498	D0PRB4
Tri a 32.0101					Tri a 32.0101		Q6W8Q2
Tri a DH	#126	Dehydrin	Recombinant	Dot blot	NA	EU584500	D0PRB6
Tri a Gliadin		Gliadin fraction	Natural	ISAC 103	Tri a Gliadin		NA
Tri a GST	#38	Glutathione-S-transferases	Recombinant	Dot blot	NA	EU584497	Q8RW02
Tri a 39	#10	Serine protease inhibitor	Recombinant	Dot blot	NA	122065237	P82977
Tri a Trx	#37	Thioredoxin	Recombinant	Dot blot	NA	EU584496	Q8GVD3

Allergens used in the study are with a grey shaded background.

*Allergen names following the Allergome nomenclature (<http://www.allergome.org>).

†Official allergen nomenclature of WHO-IUIS allergen nomenclature subcommittee.

‡Accession numbers to GeneBank (<http://www.ncbi.nlm.nih.gov/nuccore>) and Uniprot (<http://www.uniprot.org>) databases.

0.01 as $p < 0.01$. Statistical analyses were performed using the R statistical package (<http://www.r-project.org/>).

RESULTS

Characteristics of the study population according to the positivity to any occupational allergen are presented in table 2. All bakers, independent of the positivity to occupational allergens, reported an improvement or disappearance of their symptoms during weekend or holidays. The list of the work-related disorders is outlined in table 2. Gender, smoking status, age, duration of employment and family history of allergic diseases were not associated with any occupational allergen positivity, whereas the number of symptoms at work reported per person ($p < 0.01$) and personal history of allergic rhinitis were ($p < 0.05$). Mean FEV₁ values (% predicted) were lower in bakers positive to any occupational allergen. An obstructive pattern (FEV₁ value below the lower limit of normal) was observed in six subjects, whereas, among the remaining bakers with normal lung function, the methacholine bronchial challenge was positive in 36 patients.

Wheat serum specific IgE was detected in 51 workers (62.9%) whereas wheat SPT was positive in 28 subjects (34.5%). Tri a 30 was the only positive wheat ISAC allergen (three subjects, 3.6%). When considering the six dot-blot recombinant wheat allergens, a higher number of workers were identified as IgE positive ($n=22$). Tri a 32 was the most frequently detected (13 subjects; 16%). Tri a 12 was positive in five workers (6%), Tri a GST in three subjects (3.7%), and Tri a DH and Tri a 39 in two subjects (2.4%). Tri a Trx was negative in all sera. Taking into account the results obtained using wheat extracts (SPT, immunoCAP) or allergenic molecules (ISAC micro-array and the dot blot), 55 workers (67.9%) were diagnosed having a wheat specific IgE response.

Diagnosis of work-related asthma was associated with wheat SPT, wheat IgE and Tri a 32 (table 3). Work-related rhinoconjunctivitis showed a significant association with wheat IgE. Work-related dermatitis was associated with ISAC Tri a 30 and the dot-blot Tri a GST and Tri a 32. The strength of the association between work-related dermatitis and molecular wheat allergens increased when Tri a 32, Tri a GST and Tri a 30 were analysed together ($p < 0.01$).

The risk factors evaluation showed that among the personal history of non-occupational allergic diseases, seasonal rhinitis was associated with at least one positive environmental SPT (OR=3.95 (CI 1.38 to 12.32), $p < 0.01$), any ISAC positivity (OR=3.03 (CI 1.11 to 8.67), $p < 0.05$) and wheat ImmunoCAP IgE positivity (OR=3.634 (CI 1.23 to 11.91), $p < 0.05$). This association was not confirmed when we considered other occupational SPT or the wheat dot-blot allergens. A significant correlation was detected between environmental SPT positivity and IgE positivity to occupational allergens by mean of SPT and/or ImmunoCAP system (OR 4.06 (CI 1.44 to 12.07), $p < 0.01$). In multi-symptomatic patients with positive wheat ImmunoCAP IgE, the mean of wheat IgE levels significantly increased by 3.49 kU/l (β -coefficient=3.49 (CI 1.28 to 9.47), $p < 0.01$) when the number of symptoms at work increased (ie, bakers reporting the highest number of symptoms had the highest wheat IgE levels). Wheat IgE levels were significantly associated with dermatitis ($p < 0.01$).

DISCUSSION

Our study is the first attempt to correlate current diagnostic tests (SPT, specific IgE and ISAC 103 microarray system) and additional dot-blot wheat allergens with clinical phenotypes in allergic bakers. We showed that work-related asthma and rhinoconjunctivitis were independently associated with wheat IgE. We also showed that Tri a 32, Tri a GST and Tri a 30 are independently associated with work-related dermatitis, and Tri a 32 was associated with work-related asthma.

Until now, the bakers' wheat IgE reactivity to current diagnostic extracts has not provided sufficient information about the allergenic molecules involved.¹⁶ We have documented that the availability of only four wheat allergens in the ISAC panel narrows the possibility to detect allergy in bakers.¹⁸ Our results suggest that enriching the number of wheat ISAC allergens with additional allergens, such as Tri a 32 and Tri a GST, further increases detection of the number of bakers with dermatitis. Tri a 32 may also be suggested as a marker of asthma. This suggests the need of a larger commercial wheat allergen panel. However, our data need to be confirmed in a wider cohort of bakers.

Workplace

Table 2 Demographic and general characteristics of the enrolled bakers

	Any occupational allergen*				p Value
	Positive (n=56)		Negative (n=25)		
	Median	Range	Median	Range	
Number of symptoms at work per subject	3	1–9	2	1–6	<0.01
Total IgE (kU/l)	256	10.1–8293	44	2–754	<0.01
Age (years)	33	20–63	38	19–62	NS
FEV ₁ (% predicted)	103	45–130	106	81–132	NS
	N	%	N	%	NS
Gender (male)	45	80.3	18	72	NS
Bread making area employees	52	92.8	21	84	NS
Smokers	17	30.3	7	28	NS
Ex-smokers	9	16	5	20	NS
Family history of allergic diseases	25	44.6	11	44	NS
Personal history of any non-occupational allergic diseases	38	67.8	13	52	NS
Rhinitis	28	50	6	24	<0.05
Asthma	25	44.6	6	24	NS
Eczema	16	28.5	9	36	NS
Work-related rhino-conjunctivitis	52	92.8	20	80	NS
Work-related asthma	20	35.7	2	8	<0.05
Work-related dermatitis	8	14.2	1	4	NS
SPT					
Any environmental allergen	38	67.8	9	36	<0.01
Grass pollen	30	53.5	4	16	<0.01
<i>D pteronyssinus</i>	21	37.5	2	8	<0.01
ISAC 103					
Any positivity	31	55.3	8	32	0.05
Lung function					
FEV ₁ (<LLN)	5	8.9	1	4	<0.01
PD20 FEV ₁ (≤1000 µg)	30	53.5	6	24	<0.01

Significance of differences was calculated using Fisher's exact test (p value).

*According to flours (wheat, rye, barley, oat, and soy), yeast and α -amylase SPT, ImmunoCAP IgE, ISAC allergens (wheat, soy, sesame) and dot-blotted wheat allergens.FEV₁, forced expiratory volume in 1 s; LLN, lower limit of normal; NS, not significant; SPT, skin prick test.

An association between specific clinical manifestations (gastro-intestinal/cutaneous symptoms, anaphylaxis) and laboratory patterns has been identified in buckwheat allergy¹⁹ supporting our hypothesis of a different pattern of wheat allergen sensitisation in bakers suffering from diverse allergic symptoms at work.

Recent studies have confirmed that IgE of patients' sera recognise specific wheat proteins. In wheat allergy, Palosuo *et al*⁷ identified ω -5-gliadin (Tri a 19) as a major allergen in WDEIA suggesting a relationship between a specific IgE recognition and this clinical phenotype. Furthermore, levels of IgE against

Table 3 Comparison of wheat diagnostic methods according to the work-related diseases

	Current diagnostic tests in wheat allergy			Wheat dot-blotted allergens†				
	ImmunoCAP (n=51)	SPT (n=28)	ISAC† Tri a 30 (n=3)	Tri a 39 (n=2)	Tri a GST (n=3)	Tri a 12 (n=5)	Tri a 32 (n=13)	Tri a DH (n=2)
Work-related asthma								
Yes=22 (%)	20 (90.1)**	15 (68.2)**	1 (4.5)	2 (9)	1 (4.5)	1 (4.5)	8 (36.3)**	2 (9)
No=59 (%)	31 (52.5)	13 (22)	2 (3.3)	0	2 (3.3)	4 (6.7)	5 (8.5)	0
Work-related rhino-conjunctivitis								
Yes=72 (%)	49 (68)*	26 (36.1)	3 (4.1)	2 (2.7)	3 (4.1)	5 (6.9)	12 (16.6)	2 (2.7)
No=9 (%)	2 (5.4)	2 (2.8)	0	0	0	0	1 (1.3)	0
Work-related dermatitis								
Yes=9(%)	7 (77.7)	3 (33.3)	2 (22.2)*	1 (11.1)	2 (22.2)*	0	4 (44.4)*	0
No=72(%)	44 (9.7)	25 (4.1)	1 (2.7)	1 (1.3)	1 (1.3)	5 (6.9)	9 (5.5)	2 (2.7)

Absolute frequencies and frequencies in percentage value (in parentheses).

Significance of differences was calculated using Fisher's exact test *p<0.05, **p<0.01.

†ISAC Tri a 19.101; Tri a 18; Tri a Gliadin and dot blot Tri a Trx.

‡Did not show any positivity.

SPT, skin prick test.

ω -5-gliadin have been associated with the severity of the reactions at oral challenge.²⁰ None of our bakers reported symptoms after eating wheat-derived products and Tri a 19 was negative in all cases suggesting that the oral route was not involved in baker's wheat allergy. As confirmed by our results, neither ISAC Tri a 18 or Tri a Gliadin has been reported to be associated with baker allergy.

Our six dot-blotted wheat allergens have been also evaluated also by Constantin *et al*¹¹ who showed that among bakers with occupational asthma, Tri a DH, Tri a 12, and Tri a 39 were the most frequently detected allergens. Due to differences in the study population and diagnostic protocol, our results do not confirm the same reactivity profile.

Recently, Sander *et al*¹⁰ showed in bakers who reported occupational eye, nasal and respiratory symptoms that Tri a 30, Tri a 12 and Tri a 19 were positive in 15%, 2.5% and 2.5% of cases, respectively. As in our study, a specific inhalation challenge test was not performed. Our bakers were positive only to Tri a 12 (9.8%) and Tri a 30 (5.8%). Differences in the sensitisation profile might have different explanations: different cut-off selection of wheat IgE levels (0.35 kU/l in our bakers vs 0.5 kU/l as reported by Sander *et al*) and different occupational symptoms (our bakers reported also dermatitis at work). The geographical variability of the various wheat cultivars²¹ cannot be excluded in determining the frequency of sensitisation, specially to profilin (Tri a 12).²²

Recently, Tri a 32, Tri a GST and Tri a DH have been suggested to be relevant wheat allergens in respiratory wheat allergy.⁸ Other wheat allergens such as wheat water-soluble proteins of the α -amylase inhibitor family,²³ wheat flour peroxidase²⁴ and wheat prolamins,²⁵ thioredoxins,²⁶ thaumatin-like protein²⁷ and lipid transfer protein²⁸ may be also relevant in baker's asthma. Until now, the limited number of the available allergenic components to resolve wheat allergy diagnosis does not confer an advantage over currently available specific IgE detection to identify the majority of the allergic bakers.

High wheat IgE levels are good predictors for a positive challenge test in bakers with nasal and respiratory symptoms.²⁹ This statement, however, cannot be confirmed by our results since most of the bakers in our study population were multi-symptomatic. Nevertheless, we showed that bakers with dermatitis had significantly high wheat IgE levels. The involvement of a wide body area, such as the skin, or the presence of comorbidities may explain this association. However, prospective studies are needed to correlate wheat IgE variations to the onset and the severity of the different allergic symptoms.

Our results confirm that seasonal rhino-conjunctivitis and SPT positivity to common allergens are significantly associated with IgE positivity to occupational allergens³⁰ and that a history of seasonal rhino-conjunctivitis is associated with ISAC microarray positivity.¹⁶ By combining in a single test the ISAC allergens and the dot-blotted wheat allergens it may be possible to draw the environmental and occupational allergic profile in bakers.

Our bakers showed a different prevalence of sensitisation according to the diagnostic tests used. The discrepancy between wheat SPT and specific IgE positivity might be due to the different wheat protein content in the diagnostic preparations. van Kampen *et al*³ documented the wide variability of allergen concentrations by comparing wheat SPT of different producers. For this reason, we performed more than one test because of the lack of a large panel of occupational allergens. Among our bakers negative to all the diagnostic tests, only one was detected by means of specific IgE to the other occupational allergens, confirming that wheat is the major allergen in baker's allergy.

However, it could not be excluded that other allergen sources in bakeries such as buckwheat flour,³¹ lupine flours³² and other enzyme improvers³³ may be involved.

As reported in literature,³⁴ our data confirm that the sensitisation process can explain only some occupational symptoms. Bakery flour dust is a complex mixture of protein, organic materials and contaminants, such as bacterial endotoxin,³⁵ which can provoke in some cases work-related respiratory symptoms by an inflammatory or irritant reaction without the involvement of an allergic pathway.³⁴ However, endotoxin exposure in bakeries has not been evaluated yet; thus, we cannot exclude its role in the onset of symptoms at work. In our bakers not sensitised to any occupational allergens, the role of atopy in the onset of symptoms at work might be excluded because of the low level of total IgE and the low prevalence of sensitisation to environmental allergens. The hypothesis of an irritant response has been also supported by the occupational history, and the characteristics of symptoms at work and their relationship with flour exposure.

One potential limitation of this study is the lack of a specific inhalation challenge test because it was not available in our facility. This test has been often considered the 'gold standard' for the diagnosis of occupational asthma. However, specific challenge tests are time-consuming; thus, they are not well accepted by employees and false-positive and false-negative tests can be found.³⁶ Further studies are necessary to define the role of wheat allergenic components in allergic bakers by specific challenge. Another limitation of this study is the lack of a group of bakers without symptoms in addition to the study group in order to confirm our results.

In conclusion, our study confirms that atopy is very common among bakers reporting occupational allergic symptoms and that the sensitivity of commercial wheat SPT preparations is still limited in baker's allergy. Wheat IgE levels and specific wheat allergens may be associated with occupational phenotypes, such as dermatitis. However, the role of molecular wheat allergens in other occupational diseases needs to be confirmed by challenge test with specific wheat allergens. In bakers, a better definition of the wheat allergic phenotype may be helpful for health surveillance purposes or during the patient's follow-up.

Contributors MO, AM, CAB and RV conceived and designed the study. PP, SP, GZ, RF and DZ carried out the assays and participated in the interpretation of the data. GM and LX performed the statistical analysis. MO, CAB, AM and SP drafted and wrote the manuscript.

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Competing interests MO, CAB, PP, SP, GM, RF, DZ, GZ, LX and AM have no conflict of interest. RV has acted as a paid consultant for Phadia, Uppsala, Sweden.

Patient consent Obtained.

Ethics approval Local Ethical Committee of Verona, Italy.

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Workplace

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