

Letter to the Editor

Component-resolved diagnosis of wheat flour allergy in baker's asthma*To the Editor:*

Occupational asthma accounts for more than 10% of all cases of asthma in adults, and baker's asthma (BA) is the leading cause of occupational respiratory disease in Western countries.¹ Such occupational allergic respiratory disorders are often misdiagnosed, with significant legal, economic, and health impacts for affected patients.^{1,2} The incidence of BA among young bakers has been reported to range from 0.3 to 2.4 cases per 1000 person-year, and an increasing number of asthma cases are being reported among supermarket bakery workers.³

In recent years, some studies aimed to determine the panel of wheat allergens for diagnosing patients with BA to avoid specific bronchial challenge with wheat due to its potential risk and technical requirements.^{4,5} Regarding *in vitro* diagnosis (specific IgE assays), all known diagnostic approaches have poor predictability and specificity.^{1,2} The introduction of microarray techniques featuring a large panel of purified allergens has been a major advance in the diagnosis of allergic diseases.^{6,7} However, this technique has been hardly applied to the diagnosis of patients with occupational asthma caused by wheat.^{7,8}

In the present study, the allergen profiles of patients with BA from 3 different regions in Spain (Madrid, Malaga, and Valladolid) with a relevant bakery industry were characterized. Forty-five bakers with respiratory symptoms (nasal and bronchial) due to occupational exposure to wheat flour, and with confirmed diagnosis of occupational asthma by positive skin prick test (SPT) result and positive bronchial challenge test result with an in-house wheat extract, were recruited for the study (BA group). Clinical characteristics of patients are summarized in Table I. Sex distribution was similar among centers, with a similar median age and range. Sensitization to aeroallergens was tested by means of

SPT, with a similar percentage of atopy among regions (Madrid 88%, Malaga 75%, and Valladolid 89%; $P > .05$). In terms of specific sensitization, the most common allergens in Madrid and Valladolid were pollens. The prevalence of sensitization to grass pollen in Valladolid was 70%, significantly higher than in the other regions ($P > .05$; 2-way ANOVA with the Tukey multiple comparisons test). House-dust mites were the most prevalent aeroallergens in Malaga and were significantly higher than in the rest of regions ($P < .05$; 2-way ANOVA with the Tukey multiple comparisons test). Regarding food allergy, less than half of the patients were sensitized to foodstuffs as determined by SPT and clinical history.

In addition, 2 control groups from the same 3 regions were recruited for the study: (a) subjects with seasonal allergic rhinitis (confirmed by positive SPT result to pollen allergens), no respiratory sensitization to wheat flour (no symptoms and negative SPT result to wheat flour extract), and with good tolerance to wheat ingestion ($n = 41$: Madrid $n = 21$, Malaga $n = 10$, Valladolid $n = 10$) (patients with seasonal allergic rhinitis [SAR]); (b) subjects with allergy to wheat through ingestion demonstrated by a clear clinical history of allergic reaction on wheat intake and confirmed by positive SPT result to wheat flour extract, but with no symptoms of SAR ($n = 9$) (patients with wheat food allergy [WFA]).

Individual sera from the 45 patients with BA were incubated with the wheat allergen microarray (see Table E1 in this article's Online Repository at www.jacionline.org). Twelve wheat allergens associated with BA were purified from natural sources (Fig 1, A; WDAI-0.19, WDAI-0.53, WTAI-CM1, WTAI-CM2, WTAI-CM3, WTAI-CM16, WTAI-CM17, Tri a 14, profilin, ω -5-gliadin, Tri a Bd 36, and Tri a TLP) and printed on the allergen microarray to study the sensitization profile of patients with confirmed BA diagnosis (see the Methods section in this article's Online Repository at www.jacionline.org). All purified proteins were identified by N-terminal amino acid-sequencing

TABLE I. Clinical data of patients with BA included in the study

Location	Median age (y) (range)	Sex (M/F)	SPT wheat extract (positive)	Bronchial challenge test (positive)	Aeroallergen sensitization (SPT)	Food allergy (SPT)
Madrid $n = 17$	39.2 (28-64)	16/1	17/17	17/17	+ Any SPT 88% Olive 35% Grass 29% Dust mites 35% Alternaria 11.7% Alpha-amylase 17.6%	53% No 47% Yes 18% Nuts 6% Barley 6% Egg 6% Kiwi
Malaga $n = 10$	38 (19-60)	8/2	10/10	10/10	+ Any SPT 75% Olive 37.5% Grass 37.5% Dust mites 62.5% Alternaria 12.5% Alpha-amylase 12%	60% No 40% Yes 50% Peach 25% Nuts 25% Spices 25% Barley
Valladolid $n = 18$	31 (19-66)	14/4	18/18	18/18	+ Any SPT 89% Olive 50% Grass 70% Dust mites 20% Alternaria 10% Alpha-amylase 22.4%	62% No 38% Yes 50% Barley 25% Apple 25% Peach

F, Female; M, male.

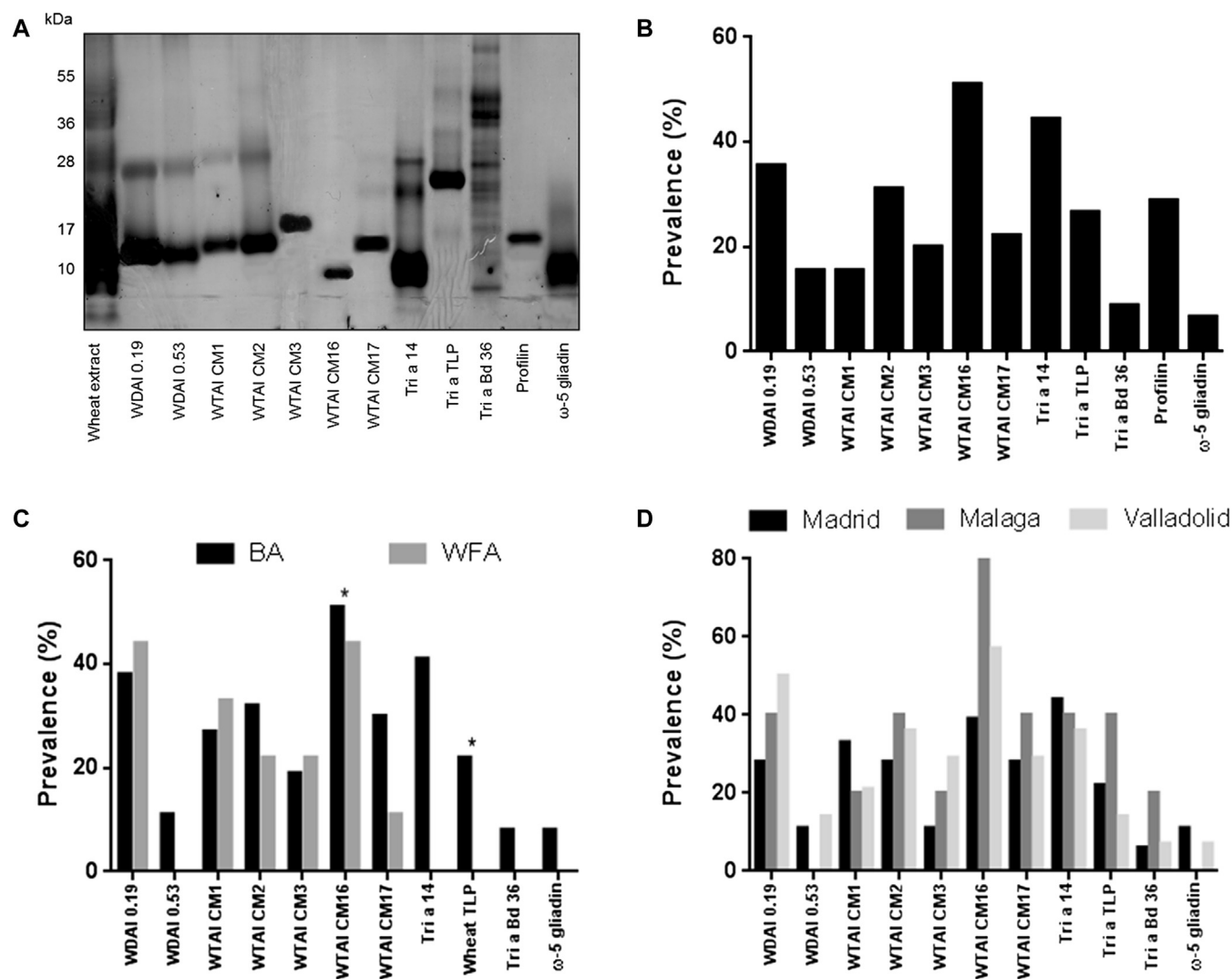


FIG 1. A, SDS-PAGE and silver staining of wheat extract and the purified wheat allergens. B, Prevalence of wheat allergens associated with BA in the whole study population. C, Prevalence of individual wheat allergens in patients with BA and WFA. * $P < .05$. D, Prevalence of individual wheat allergens in patients with BA from Madrid, Malaga, and Valladolid.

and mass spectrometry (MALDI-TOF-TOF Autoflex, Bruker, Bremen, Germany) in the Proteomic Service of CIB, CSIC (Madrid, Spain).

In the present study, more than 80% of these patients recognized some of the printed allergens. The highest prevalence of IgE binding was observed for WTAI-CM16 (54% positivity) and Tri a 14 (45% positivity). These allergens were significantly more prevalent in patients with BA than in those from the control groups (WTAI-CM16, $P = .0093$; and Tri a 14, $P = .0251$; 2-way ANOVA with the Tukey multiple comparisons test), covering 64% of the studied population (Fig 1, B). In contrast, the rest of the purified allergens were recognized by 10% to 30% of the subjects.

Receiver operating characteristic curves were calculated comparing SPT and microarray response to Tri a 14 and WTAI-CM16 as a way to study the validity of the present diagnostic tool. The obtained area under the curve was 0.722 for Tri a 14 and

0.622 for WTAI-CM16, respectively, suggesting that the obtained values are within the standard when diagnostic methods are compared with this test.

In recent years, some studies aimed to determine the panel of wheat allergens for diagnosing patients with BA to avoid specific bronchial challenge with wheat due to its potential risk and technical requirements. Sander et al⁹ produced a panel of 17 recombinant allergens associated with BA. However, the frequencies of sensitization observed were low and they could not detect any major allergen. The discrepancy of our results with those reported by Sander et al may be due to the use of recombinant allergens as acknowledged by the authors in their article. Sotkovsky et al¹⁰ studied the IgE binding of wheat allergens using natural forms. In this study, more than 20 allergens were identified but only Tri a 14, Tri a TLP, and tritin were recognized by more than 50% of sensitized subjects (64%, 100%, and 100%, respectively), being thus considered as major allergens.

In our study, statistically significant differences were found in the allergen recognition among subjects of BA, SAR, and WFA groups (Fig 1, B and C; $P = .0003$; ordinary 1-way ANOVA). Nevertheless, no statistical differences were observed among the sensitization prevalence to wheat allergens in patients with BA from the 3 regions ($P = .6417$; ordinary 1-way ANOVA; Fig 1, D). This indicates that the wheat allergen profile of our studied BA population was not influenced by environmental allergen patterns (see Fig E1 in this article's Online Repository at www.jacionline.org).

Another fact worth mentioning is that Tri a 14 was recognized only by patients with BA (44%), but not by those who were diagnosed with WFA or SAR. In previous studies by our group, more than 60% of the Spanish patients with BA reacted, both *in vitro* and *in vivo*, to Tri a 14.¹¹ Similarly, in the work by Sotkovsky et al,¹⁰ 64% of the patients with BA showed IgE binding to Tri a 14 in a Czechoslovakian population. Although Tri a 14 shows a low sensitivity in the diagnosis of BA, because it is recognized by less than 50% of the patients with BA, it shows a high specificity in the diagnosis of this disorder. Hence, Tri a 14 is a major allergen involved in the development of BA.

Summarizing, BA is an allergic disorder caused by the inhalation of wheat flour allergens, which does not seem to be influenced by local environmental allergens (pollen), and it appears to be associated with a specific profile of allergen sensitization that is different from that observed in patients with wheat-induced food allergy. In addition, Tri a 14 seems to be exclusively associated with wheat-induced allergy by inhalation having a high diagnostic value.

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METHODS

Selection and purification of allergens

Twelve wheat allergens were purified from natural sources following a previously published method (see [Table E1](#)). The proteins were chosen on the basis of their previously described allergenic properties and their relationship with BA. All purified proteins were identified by N-terminal amino acid-sequencing and mass spectrometry (MALDI-TOF-TOF Autoflex) in the Proteomic Service of CIB, CSIC. In addition, every purified wheat protein (5 μ g) was visualized on silver-stained SDS-PAGE (see [Fig 1](#)).

Production of allergen microarray and immunoassay

The allergen microarray for BA was produced as previously described. Purified proteins (0.25 and 0.125 mg/mL in $1\times$ Protein Binding Buffer [GE Healthcare, Uppsala, Sweden] containing 0.02% Tween 20) were applied on epoxy-activated glass slides (TeleChem International, Sunnyvale, Calif) with

16 microarrays per slide, using a MicroGrid II TAS arrayer (BioRobotics, Genomic Solutions, Ann Arbor, Mich). Each array well was incubated for 1 hour at room temperature with blocking solution (Sigma, St Louis, Mo) and then incubated overnight with 80 μ L undiluted serum at 4°C. To detect bound IgE antibodies, the slides were incubated for 1 hour at room temperature with diluted 1:100 antihuman IgE PE-DY 647-labeled (Thermo Scientific, Rockford, Ill). Three spots from every sample were included in each array, and 2 replicates of each assay were performed (Pearson correlation = .955; $P < .0001$).

The IgE binding of each allergen spot was calculated as the final fluorescence intensity, obtained by subtracting the local background (B) from the observed value and the fluorescence intensity from the blank control, measured by GenePix software (Molecular Devices, St Grégoirecédex, France) by applying the equation: $I = (F_{645} - B)_{\text{sample}} - (F_{645} - B)_{\text{blank}}$. Fluorescence intensity levels of more than 200 units were considered to be positive (mean + $3 \times$ SD of negative control spots, those containing blocking solution).

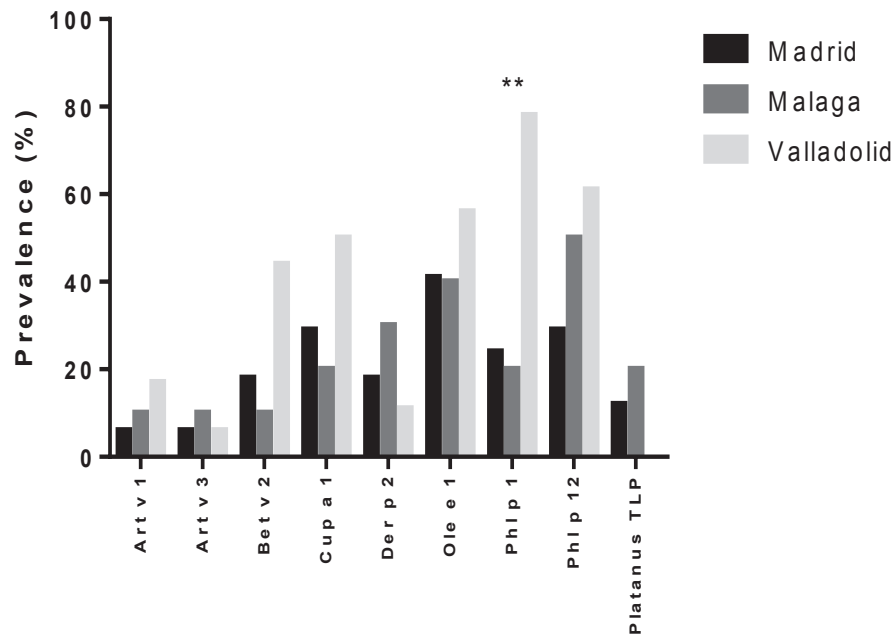


FIG E1. Prevalence of sensitization to major inhalatory allergens in subjects with BA from 3 different regions. ** $P < .05$.

TABLE E1. Purified wheat proteins included in the wheat allergen microarray

Short or abbreviated name of wheat protein	Name of wheat protein	Allergen (IUIS*)
WDAI-0.19	Wheat dimeric α -amylase inhibitor 0.19	Tri a 28.0101*
WDAI-0.53	Wheat dimeric α -amylase inhibitor 0.19	Tri a 28
WTAI-CM1	Wheat tetrameric α -amylase inhibitor CM1	Tri a 29.0101*
WTAI-CM2	Wheat tetrameric α -amylase inhibitor CM2	Tri a 29.0201
WTAI-CM3	Wheat tetrameric α -amylase inhibitor CM3	Tri a 30.0101*
WTAI-CM16	Wheat tetrameric α -amylase inhibitor 16	Tri a CM16
WTAI-CM17	Wheat tetrameric α -amylase inhibitor CM17	-
nsLTP	Nonspecific lipid-transfer protein type I	Tri a 14.0201*
Profilin	Profilin	Pho d 2†
ω -5-Gliadin	Omega-5-gliadin	Tri a 19
Tri a Bd 36	Peroxidase	Tri a Bd 36
Tri a TLP	Thaumatococcus-like protein	Tri a TLP

IUIS, International Union of Immunological Societies.

*Name accepted by IUIS.

†The profilin used in this work was Pho d 2, the pollen palm profilin.