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Prevalence of work-related symptoms and serum-specific antibodies to wheat flour in exposed workers in the bakery industry

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Summary

Background: Although baker's asthma (BA) is a common occupational asthma, there have been few reports on this disease in Korean subjects.

Objectives: We evaluated the prevalence of serum-specific IgE, IgG1, and IgG4 antibodies in relation to work-related respiratory symptoms in a single industrial bakery.

Methods: Three hundred and ninety-two bakery workers were administered and taken a questionnaire regarding respiratory symptoms. For symptomatic workers, the methacholine bronchial challenge test and specific bronchoprovocation tests with wheat extracts were carried out. Skin prick tests were performed and serum-specific IgE, IgG1, and IgG4 antibodies to wheat flour were detected. The IgE- and IgG4-binding components were identified by immunoblotting.

Results: Sixty-seven workers (17.1%) complained of work-related upper and lower respiratory symptoms. The prevalence of BA based on positive bronchoprovocation test results was 1.5%. The sensitization rate to wheat flour was 5.9% by skin prick test and 6.5% by ELISA, and was closely associated with the presence of atopy and work-related lower respiratory symptoms ($P < 0.001$ for both). IgE immunoblotting revealed six major IgE-binding components (27, 31, 36, 43, 54, and 72 kDa). The presence of wheat-specific IgG1 and IgG4 antibodies was found to be significantly associated with exposure intensity ($P < 0.05$ for both).

Conclusions: The overall prevalence of wheat sensitization in a Korean bakery was 5.9%. We confirmed that an IgE-mediated response is the major pathogenic mechanism for the

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induction of work-related symptoms in wheat-exposed workers. Wheat-specific IgG antibodies may represent current or previous exposure to wheat dust.

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Introduction

Baker's asthma (BA) and rhinitis are some of the most common occupational respiratory disorders in western countries; in fact, there have been several epidemiological reports on BA.¹⁻⁴ The prevalence of BA has increased in Asian countries in recent years. In terms of pathogenesis, BA is an IgE-mediated response to the inhalation of wheat flour,⁵ although a possible role for specific IgG has been suggested.^{6,7} Although wheat flour is the major source of allergen, several other bakery allergens, including rye, barley, soya flour, and other additive components, can induce the formation of specific IgE antibodies. To date, few studies have been evaluated the clinical significance of serum-specific IgE and IgG antibodies or compared antigen-binding components in association with work-related symptoms in bakers in the same working environment. Thus, we investigated the clinical and immunological characteristics of 392 exposed subjects working in the largest industrial bakery in Korea.

Methods

Study subjects

A questionnaire was used to screen for work-related upper and lower respiratory symptoms in 392 workers from a single industrial site in July 2006. All workers were asked to complete the questionnaire, the questions regarding respiratory symptoms included whether subjects had experienced upper respiratory symptoms such as nasal itching, runny nose, sneezing, or congestion, as well as lower respiratory symptoms, such as cough, sputum, shortness of breath or wheezing. The subjects who indicated that their symptoms were aggravated during the work, but improved after the work or during holidays were defined as having work-related respiratory symptoms. The protocols used in this study were reviewed and approved by the Ajou University Institute Review Board. Informed consent was obtained from each participant.

Skin prick test and measurement of total IgE

All 392 workers were given a skin prick test, which included common inhalant allergens, i.e., tree mixture, grass mixture, mugwort, ragweed, cat fur, dog fur, *Dermatophagoides pteronyssinus*, *D. farinae*, and *Alternaria* (Bencard, Bretford, UK), and baker's allergen extracts, including wheat, rye, baker's yeast (collected from the workplace and prepared as described below at a concentrations of 2 mg/ml), egg (Bencard), α -amylase (from *Aspergillus* spp.; Sigma-Aldrich, St. Louis, MO, USA) and storage mite (*Tyrophagus putrescentiae*; Allergopharma, Reinbek, Germany).

The results of the skin prick tests are reported as the ratio of the mean wheal diameter of the allergen to histamine (A/H ratio). For $A/H \geq 1$, the reaction was defined as positive. Atopy was defined for subjects who had more than one positive response to common inhalant allergens on the skin prick test. Serum total IgE levels were measured using the immunoCAP system (Phadia AB, Uppsala, Sweden).

Preparation of baker's allergens

Baker's allergens were extracted with phosphate-buffered saline [PBS (pH 7.5), 1:5 wt/vol] overnight at 4°C. The protein concentrations in the supernatants of wheat, rye, and dry yeast were determined by the Bradford method using a protein assay kit (Bio-Rad, Hercules, CA, USA). These proteins were used for enzyme-linked immunosorbent assay (ELISA) and immunoblotting. For the skin prick test, the supernatants were mixed with sterile glycerin.

Bronchoprovocation tests with methacholine and wheat flour extracts

The subjects with work-related lower respiratory symptoms were selected for methacholine bronchoprovocation testing. Airway responsiveness to methacholine was tested during each subject's workday using the five-breath dosimeter protocol. Briefly, normal saline was administered from a nebulizer 646 connected to a dosimeter (Devilbiss Co., Doylestown, PA, USA). The subject was asked to breathe 5 times using dosimeter. The concentrations of inhaled methacholine were ranged from 1.25 to 25 mg/ml. The pulmonary function tests including forced expiratory volume in 1 s (FEV_1) and forced expiratory flow at 25-75% (FEF_{25-75}) were measured with a spirometer (Jaeger; MasterScope PC, Hoechberg, Germany) 10 min after each inhalation. The concentration of inhaled methacholine that causes a 20% fall in FEV_1 (PC_{20}) was measured.

The subjects with a positive result were subsequently given bronchoprovocation tests with wheat extracts according to a previously described procedure.⁸ Briefly, normal saline was administered from a nebulizer 646 connected to a dosimeter (Devilbiss Co). The subject was asked to breathe 5 times using dosimeter. The concentrations of inhaled wheat extracts were ranged from 1 μ g/ml to 1 mg/ml, which were decided as the previous results of intradermal tests with wheat extracts. The pulmonary function tests including FEV_1 and FEF_{25-75} were measured with a spirometer (Jaeger) before and 10 min after each inhalation. Then, the FEV_1 and FEF_{25-75} were measured every 10 min during the first hour, and pulmonary function tests were performed every hour for 7 h after the challenge. When the FEV_1 level decreased more than 20% from the baseline value, it was regarded as a positive response.

ELISA for specific IgE, IgG1, and IgG4 antibodies to wheat extracts

The presence of specific antibodies to wheat extracts was determined by ELISA, as described previously.⁹ Fifty microliters of undiluted serum from each worker and from each of 60 unexposed healthy controls with negative responses to common inhaled allergens and baker's allergen were used to detect specific IgE, IgG1 and IgG4 antibodies, which was measured using 1:10 dilutions of serum with biotin-labeled goat anti-human IgE antibodies (1:1000 dilution; Vector Laboratories Inc., Burlingame, CA, USA) and biotin-labeled goat anti-human IgG1 antibodies (1:1000 dilution; Sigma-Aldrich) or IgG4 antibodies (1:1000 dilution; Sigma-Aldrich), respectively. Positive cut-off values were derived from the mean plus three standard deviations of the unexposed controls.

SDS-PAGE and immunoblotting

Workers whose serum samples contained elevated titers of serum-specific IgE and IgG4 antibodies to wheat flour and who suffered from BA or rhinitis, as confirmed by provocation challenge tests, were enrolled for both IgE and IgG4 immunoblotting. The subjects were classified into three groups according to their ELISA results: (1) those who had both serum-specific IgE and IgG4 antibodies to wheat flour; (2) those who had high levels of serum-specific IgE without IgG4 antibodies to wheat flour; and (3) those who had high levels of serum-specific IgG4 without IgE antibodies to wheat flour.

SDS-PAGE and immunoblotting were performed as described previously using wheat flour extracts (30 µg/well).⁹

Dust exposure assessment

The workers were equipped with personal inhalable-dust samplers (IOM inhalable-dust sampler; SKC Inc., Eighty Four, PA, USA) to assess the environmental dust concentrations in their current departments. Representatives from each department wore personal inhalable-dust samplers, and dust samples were collected in the workers' breathing zones during their regular shifts (8h). In total, 87 separate dust samples were collected from each department, and the dust concentration was analyzed for each exposure group.

Statistical analyses

All data are expressed as the mean ± standard error. SPSS software (version 11.05; Chicago, IL, USA) was used to perform all statistical analyses.

Results

Subjects' characteristics and clinical findings

The mean age of the 392 workers was 34.85 ± 7.68 years, and the mean exposure period in the bakery was 3.92 ± 3.48 years (Table 1). Of the 392 workers, 67 (17.1%) had work-related respiratory symptoms; 53 (13.5%) exhibited lower

Table 1 Clinical characteristics of the bakery workers (N = 392).

	N (%)
Gender	
Male	224 (57.1)
Female	168 (42.9)
Smoking	159 (40.7)
Atopy	134 (34.8)
History of food allergy	40 (10.3)
History of drug allergy	7 (1.9)
Work-related symptoms	67 (17.1)
Lower respiratory symptoms	53 (13.5)
IgE antibodies results (N = 381)	
Serum total IgE > 114 kU/l	154 (40.4)
Specific IgE to wheat flour	25 (6.6)
Specific IgE to rye	24 (6.1)
Specific IgE to fungal α-amylase	5 (1.3)
Specific IgE to any baker's allergen	47 (12.3)
Skin prick test (N = 387)	
Wheat flour	23 (5.9)
Rye	9 (2.3)
Yeast	15 (3.9)
Fungal α-amylase	2 (0.5)
Any baker's allergen	48 (12.4)

respiratory symptoms. Elevated total IgE levels (> 114 kU/l) were observed in 154 (40.4%) of the workers. Positive skin prick tests ($A/H \geq 1$) to wheat flour were found in 23 (5.9%) workers, and high levels of serum-specific IgE antibodies to wheat flour were detected in 25 (6.5%) workers. The prevalence of positive skin prick tests and specific IgE antibodies to any baker's allergen was 12.4% and 12.3%, respectively. Atopy was detected in 34.8% of the subjects, whereas the prevalence of food and drug allergies was 10.3% and 1.9%, respectively. One hundred and fifty-nine (40.7%) of the subjects were smokers.

Bronchoprovocation test with wheat flour extracts

A methacholine bronchial challenge test was performed in 16 workers with work-related lower respiratory symptoms because 37 of the subjects refused to participate in any additional studies. Of these 16 subjects, seven had a positive result with a mean PC₂₀ of 7.30 ± 8.35 mg/ml (ranging from <1.25 to 25 mg/ml). Among these seven positive responders, two had increased airway hyperresponsiveness (PC₂₀ = 1.25 mg/ml, for both), three had intermediate response (2.5 < PC₂₀ < 8 mg/ml), and the others, two had mild response (PC₂₀ ≥ 8 mg/ml). These seven subjects were participated in a specific bronchoprovocation test using wheat extracts. Six of the subjects had positive asthmatic responses. Among them, two subjects showed severe anaphylactic responses as well as asthmatic responses, and the other four subjects showed early asthmatic reactions. Asthmatic reactions characterized by a reduction in FEV₁ of > 20%, but which could be recovered by inhalation

of salbutamol, were diagnosed as BA. Based on these results, the prevalence of BA was 1.53%.

Measurement of wheat dust concentration and its relationship with worker symptoms

The workers were split into three groups based on the extent of exposure to wheat flour (Table 2A).¹⁰ The subjects in Group III had the highest wheat dust exposure levels (geographic mean, 3.04 mg/m³); this group consisted of workers in the mixing, weighing, and sieving departments. For Group II, which comprised workers engaging in foaming, baking, and decoration, the geographic mean was 1.16 mg/m³. The remaining workers, who did not work in the main production area, were assigned to Group I. The geographic mean of this group was 0.01 mg/m³.

The workers with intermediate to high levels of exposure to wheat dust (Groups II and III) had a higher risk of lower respiratory symptoms and an increased prevalence of serum-specific IgG1 and IgG4 antibodies than did workers in Group I (Table 2B). However, no significant association was found with specific IgE antibodies.

Relationships with specific IgE antibodies to wheat flour

Atopy status was significantly associated with skin reactivity and positive specific IgE antibodies to wheat ($P < 0.001$ and $P < 0.05$, respectively; Table 3). The intensity of exposure to wheat dust was not associated with the sensitization rate. The subjects with work-related lower respiratory symptoms had significantly higher sensitization rates to wheat than subjects without work-related respiratory symptoms ($P < 0.001$).

Relationship between the prevalence of specific IgG1 and IgG4 antibodies to wheat flour and clinical or exposure factors

Significant associations were found between the prevalence of serum-specific IgG1 or IgG4 antibodies to wheat flour and the intensity of wheat flour exposure. The prevalence of serum-specific IgG1 or IgG4 antibodies to wheat was significantly higher in the heavily exposed group than in the other groups ($P < 0.001$ for both; Table 4). Similarly, the mean level of specific IgG1 or IgG4 antibodies to wheat flour was significantly higher in the group subjected to intermediate to high levels of exposure (Groups II and III) than in the group that received limited exposure (Group I; $P < 0.001$). The prevalence of serum-specific IgG4 was associated with the presence of work-related lower respiratory symptoms ($P < 0.05$).

Identification of IgE- and IgG4-binding components to wheat flour

IgE immunoblotting using wheat flour extracts and serum from workers who had specific IgE antibodies to wheat flour revealed more than 10 IgE-binding components, including six major components: 27 kDa (62.5%), 31 kDa (87.5%), 36 kDa (50%), 43 kDa (75%), 54 kDa (75%), and 72 kDa (50%); Figure 1. The most frequently observed binding component was a 31-kDa protein, which was noted in 87.5% of the sera tested. IgE and IgG4 immunoblotting using the serum of subjects positive for BA and/or rhinitis demonstrated that these sera contained six identical components, i.e., 11, 17, 21, 54, and 96 kDa, bound to both IgE and IgG4 antibodies (Figure 2).

Table 2 (A) Concentration of wheat dust (mg/m³) in three different areas of the bakery; (B) association of clinical parameters, skin prick test results, and specific IgE antibodies to wheat flour with exposure.

Exposure category		Number of workers	Number of samples	Geographic mean	Range
I	Minimal	112	22	0.01	0.00–0.35
II	Intermediate	129	28	1.16	0.02–5.97
III	High	144	37	3.04	0.07–11.27
		<i>Minimal (Group I)</i>	<i>Intermediate to high (Groups II and III)</i>	<i>P</i>	
Work-related symptoms		20/67	47/67	0.494	
Lower respiratory symptoms		11/53	42/53	< 0.05	
Skin prick test to wheat		6/23	17/23	0.459	
Specific IgE to wheat		6/25	19/25	0.378	
Specific IgG1 to wheat		8/80	72/80	< 0.001	
Specific IgG4 to wheat		5/56	51/56	< 0.001	

Group I included packaging, supplies, development, shipping, account, distribution, and other office workers. Group II included foaming, baking, decoration, and production managers. Group III included mixing, weighing, and dividing departments.

A positive skin prick test is defined as an *A/H* ratio > 1.

A positive result for specific IgE antibodies is defined as a level greater than or equal to the mean plus three standard deviations of the level in non-atopic healthy controls.

Table 3 Association of clinical parameters with the skin prick test results and specific IgE antibodies to wheat flour.

	Skin prick test		Specific IgE	
	Positive	<i>P</i>	Positive	<i>P</i>
Presence of work-related respiratory symptoms	15/67	<0.001	13/65	0.060
Lower respiratory symptoms	10/53	<0.01	13/52	<0.01
Atopy	21/134	<0.001	14/131	<0.05
Highly exposed group	20/142	0.412	13/142	0.447
Smoking	7/59	0.125	8/57	0.590
Other exposed allergens				
House dust mite	17/108	<0.005	10/105	0.171
Rye	11/12	<0.001	8/12	<0.001
Yeast	2/15	0.357	2/14	0.237
Storage mite	4/18	0.052	1/17	1.000

A positive skin prick test is defined as an *A/H* ratio > 1.

A positive result for specific IgE antibodies is defined as a level greater than or equal to the mean plus three standard deviations of the level in non-atopic healthy controls.

Table 4 Association of clinical parameters with the prevalence of serum-specific IgG1 and IgG4 antibodies to wheat flour.

	Specific IgG1		Specific IgG4	
	Positive	<i>P</i>	Positive	<i>P</i>
Presence of work-related respiratory symptoms	20/65	0.044	19/65	<0.005
Lower respiratory symptoms	13/52	0.465	13/56	<0.05
Atopy	21/131	0.235	22/131	0.444
Highly exposed group	33/142	<0.001	28/142	<0.001
Smoking	12/57	1.000	5/57	0.237
Specific IgE to wheat flour	7/25	0.444	6/25	0.235
Other allergens				
House dust mite	17/105	0.160	15/105	1.000
Rye	3/12	0.724	4/12	0.087
Yeast	3/14	1.000	3/14	0.447
Storage mite	5/17	0.374	5/17	0.152

Discussion

BA is a common occupational disease, and wheat flour is a major causative agent of occupational asthma.^{11,12} Several epidemiological studies have reported the prevalence of BA and rhinitis among professional bakers.^{13–15} In our study population, the prevalence of rhinitis and asthmatic symptoms was 31.6% and 13.5%, respectively, which is similar to that of previous studies. However, the prevalence of BA was 1.53%, which is lower than that reported previously.¹⁶ This may be because the diagnosis of BA was based on not only self-reported symptoms and sensitization results, but also on positive responses to both the methacholine challenge and specific bronchoprovocation tests. The other reason for lower prevalence may be derived from a selection bias as only 16 subjects among 53 could undergo a methacholine bronchial challenge as they worried losing their job and believed they had no disease. Furthermore, most subjects were reluctant to advance to

the next step of wheat-specific bronchoprovocation test, when they had a negative result on methacholine challenge test.

Wheat sensitization is thought to be a major contributor to the development of BA.^{15,17,18} The prevalence of wheat flour sensitization has been variously reported as 5–15%, which is comparable to our results (5.9% by skin prick test and 6.5% by ELISA). Atopy is another risk factor for sensitization and the development of BA^{17,19} and other forms of occupational asthma.^{20,21} Therefore, sensitization to wheat flour and atopy can be used to predict the development of work-related symptoms.²²

The current intensity of exposure to occupational allergens can be a risk factor for work-related symptoms, as evidenced by the close correlation between exposure and sensitization to wheat.^{14,23,24} Workers that were in the intermediate to high-exposure group reported more asthmatic symptoms and had higher levels of wheat-specific IgG antibodies than did workers with minimal exposure.

According to our data, the workers with rye sensitization also exhibited a higher rate of wheat sensitization. Several studies have indicated high levels of cross-reactivity between wheat flour and rye flour.^{25,26} Therefore, workers who are sensitized to wheat flour may have an increased risk of sensitization to rye flour. Although storage mite is a potential causative allergen for BA, it was previously reported that the sensitization rate to storage mites was similar between bakery and control workers (12.4% vs. 10.9%; odds ratio, 1.1).¹⁹ We obtained analogous results;

sensitization to the storage mite was not associated with sensitization to wheat flour.

Several other allergens, including rye flour, α -amylase, and soy bean flour, can induce sensitization and BA.²⁷ The skin prick test results identified few positive responders to rye (9/392), yeast (12/392), and α -amylase (2/392); however, all of the rye responders had a positive response to wheat. In this study, no case of BA caused by rye, α -amylase, or other bakery allergen was identified.

Wheat flour is a typical high-molecular-weight allergen that induces IgE-mediated occupational asthma.^{5,27,28} Therefore, wheat-specific IgE may be a major contributor to the development of BA. To date, significant effort has been made to identify the IgE-binding allergenic components in wheat allergy, and allergenic components within wheat antigens have been identified.^{25,29-32} We observed more than 10 IgE-binding components, six of which are potentially major allergens. Among these, the 27- and 36-kDa proteins appear to be comparable to acyl-coA oxidase and a glycoprotein with peroxidase activity, as suggested previously.^{28,33} We noted no binding differences in comparing the symptomatic and asymptomatic sensitized subjects. These findings confirm that the IgE-mediated response is the key pathogenic mechanism for the development of BA because symptomatic workers showed positive results in the skin prick test and bronchoprovocation test, and high levels of serum-specific IgE with IgE-binding components were identified.

A few studies have suggested the possible involvement of specific IgG antibodies in the pathogenesis of BA. Among the various IgG subtypes, specific IgG4 antibodies may have roles similar to those of specific IgE antibodies in atopic patients.^{34,35} Tiikkainen et al.⁶ suggested that the levels of IgG and IgG subclasses to wheat flour in bakers reflected exposure, but were not related to any specific clinical situation. We also demonstrated that the levels of wheat-specific IgG1 and IgG4 antibodies were directly

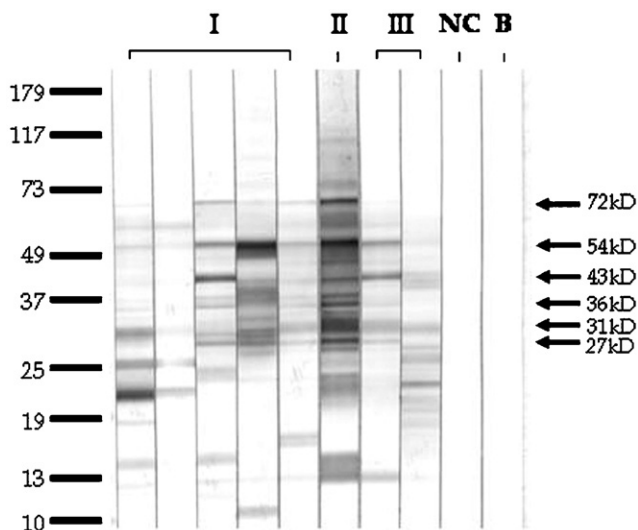


Figure 1 IgE immunoblots of sera from BA patients (I), rhinitis patients (II), asymptomatic exposed controls (AEC; III), and unexposed normal controls (NC). Six major IgE-binding components are indicated; 27, 31, 36, 43, 54, and 72 kDa. B, Blank.

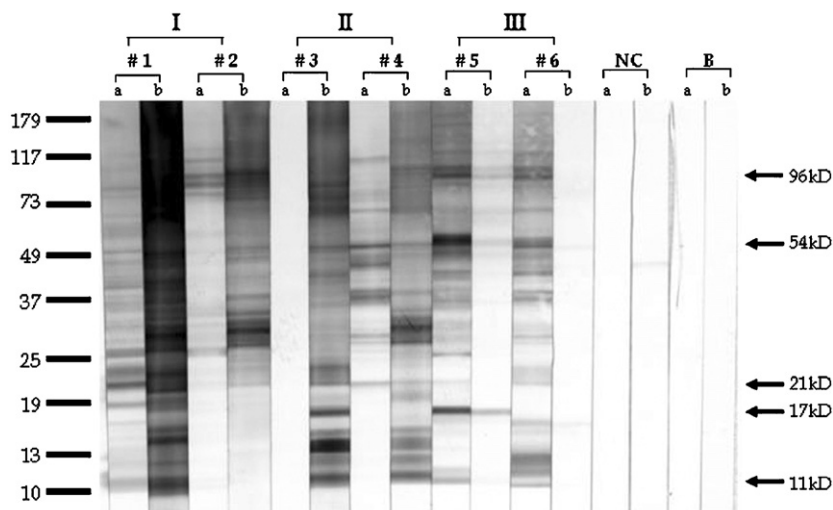


Figure 2 Comparison of the IgE- and IgG4-binding components identified in the sera of patients from three different groups and unexposed normal controls (NC). Group I includes sera from five patients with high levels of specific IgE and IgG4 antibodies to wheat extracts. Group II includes the serum of one patient with a high level of specific IgG4 antibodies to wheat extracts but no specific IgE antibodies. Group III includes sera from two patients with high levels of specific IgE antibodies to wheat extracts but no specific IgG4 antibodies. Six binding components (11, 17, 21, 54, and 96 kDa) are shared by the specific IgE and IgG4 antibodies. I, IgE+ and IgG4+; II, IgE- and IgG4+; III, IgE+ and IgG4-; #1-6 indicate individual patients. a, IgE; b, IgG4; NC, normal controls; B, blank.

correlated with the levels of exposure to wheat and the concentration of wheat dust in the workplace. Moreover, the presence of specific IgG4 antibodies was associated with the occurrence of work-related symptoms. When we compared the IgG4 and IgE immunoblots, several common binding components were noted, which suggests that IgG4 may play a role in the development of asthmatic symptoms in exposed workers who lack serum-specific IgE antibodies to wheat flour. We thus speculate that the presence of specific IgG antibodies is associated with the exposure to specific allergens.

In conclusion, the prevalence of sensitization to wheat flour and BA was 12.4% and 1.53%, respectively, within a single large bakery. We confirmed that an IgE-mediated response is the major pathogenic mechanism underlying work-related symptoms in exposed workers, and wheat-IgG may reflect exposure to wheat dust. Therefore, the detection of both serum-specific IgE and IgG antibodies to wheat flour may be a useful tool for identifying sensitized workers.

Conflict of interest statement

All the authors (Gyu-Young Hur, Dong-Hee Koh, Hyoun-Ah Kim, Han-Jung Park, Young-Min Ye, Kyoo-Sang Kim, and Hae-Sim Park) have declared that we have no conflict of interest.

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