



Evaluation of the association between sensitization to common inhalant fungi and poor asthma control

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ABSTRACT

Background: Fungi are well-known airborne allergens that are predisposing environmental factors to asthma. Few comparative studies have evaluated sensitization to common inhalant fungi in relation to poor asthma control in patients with asthma.

Objective: To evaluate the association between sensitization to individual fungi and asthma control and elucidate the characteristics of patients with poorly controlled asthma sensitized to fungi.

Methods: This cross-sectional study was performed at Showa University Hospital between September 2014 and December 2014. The specific IgE levels for several major aeroallergens, including house dust mites, Japanese cedar, various types of pollen, furry animals, or insects, were measured with a fluorescent enzyme immunoassay in 160 patients with adult asthma.

Results: Fungal sensitization was predominant in men with asthma, and it was associated with poor asthma control. Sensitization to house dust mites, Japanese cedar, pollen, furry animals, or insects was not associated with poor asthma control. Logistic regression analyses revealed that patients sensitized to *Aspergillus* and *Penicillium* had a significantly increased risk of poor asthma control. More *Penicillium* IgE–positive patients were men and pet owners compared with *Penicillium* IgE–negative patients; in addition, *Penicillium* IgE–positive patients had higher total IgE levels. The Asthma Control Test level was significantly higher in *Penicillium* IgE–positive patients than in *Penicillium* IgE–negative patients. However, there were no differences in fractional exhaled nitric oxide, forced vital capacity, and forced expiratory volume in 1 second. Finally, sensitization to *Aspergillus*, *Cladosporium*, and *Trichophyton* were positively correlated with sensitization to *Penicillium*.

Conclusion: Sensitization to fungi is predominant in men, and it is associated with poor asthma control. In particular, sensitization to *Penicillium* and *Aspergillus* is a risk factor for asthma severity. These results have potential relevance in asthma management.

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Introduction

Asthma is a complex disease caused by a combination of genetic and environmental factors. Airborne allergens are predisposing environmental factors to allergic airway diseases, including asthma. House dust mites (HDMs) are the most common allergens that induce asthma in East Asia, including Japan. Fungal spores are also important airborne allergens for people with asthma because

they exist ubiquitously indoors and outdoors. Previous studies have found that fungal sensitization is associated with an increase in the serum total IgE¹ and the development of allergy or asthma symptoms.^{2–4} Fungal growth is significantly modified by variations in the relative humidity, ambient temperature, and length of exposure to damp environments.⁵ In Japan, the climate is relatively hot and humid, particularly in the summer; thus, the condition is favorable for fungal growth. A major difference to other allergens, such as HDM, animal dander, or various types of pollen, is that fungi can colonize in the airway; hence, they can damage the lungs by producing toxins, proteases, enzymes, and volatile organic compounds. Therefore, fungi are associated with IgE-mediated allergic diseases and other pulmonary diseases, such as life-threatening

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infections, in patients using immunosuppressive agents (eg, cyclosporine, corticosteroids, and cyclophosphamide), broad-spectrum antibiotics, and indwelling catheters. Fungi also cause hypersensitivity pneumonitis, fungal sinusitis, and allergic bronchopulmonary mycosis.^{6,7}

Previous reports have found that fungal sensitization is associated with the exacerbation of asthma symptoms⁸ and an increase in emergency department visits for asthma.^{9–11} Specifically, allergic bronchopulmonary aspergillosis (ABPA), usually caused by *Aspergillus fumigatus*, is the most common concomitant disease that elicits poor control in patients with asthma. Although few population-based estimates of ABPA have been made because of uncertain diagnostic criteria, the worldwide prevalence of ABPA is estimated at 2.5% (range, 0.72%–3.5%) in adults with asthma.¹² Fungal sensitization is associated with the severity of asthma, even in patients who do not meet the definition of ABPA. Denning et al¹³ advocated the concept of severe asthma with fungal sensitization in patients who have severe asthma and the evidence of fungal sensitization but do not meet the criteria for ABPA. Although it was originally suggested that the term *severe asthma with fungal sensitization* should only be applied to adulthood, it has become evident that fungal sensitization is associated with an increase in asthma severity in childhood.¹⁴

The positivity and relevance of sensitization to airborne allergens in patients with asthma vary on the basis of environmental factors, particularly the climates where people live. The association between sensitization to individual fungi and asthma control has not been fully elucidated, particularly in Japan. In the current study, we measured 7 major fungus specific IgE in addition to other airborne allergens, including HDM, Japanese cedar, various types of pollen, furry animals, and insects, in patients with asthma in Tokyo to determine which fungal sensitization is most associated with asthma control and the characteristics of patient with poorly controlled asthma sensitized to fungi.

Methods

Patients

A total of 160 patients were randomly recruited from an asthma clinic at Showa University Hospital, Tokyo, Japan. All patients had been followed up regularly for at least 2 years at Showa University Hospital. A total of 157 patients were residents of Tokyo, and the remaining 3 patients resided in the suburban area of Tokyo. Asthma was diagnosed based on the Global Initiative for Asthma (GINA) guidelines.¹⁵ Patients with chronic obstructive lung disease or other lung diseases, poor adherence to treatment (<80%), a smoking history of more than 20 pack-years, vocal cord dysfunction, or neurologic diseases were excluded. Adherence to treatment was assessed by self-reports and each patient's prescription history. Perennial allergic rhinitis and seasonal cedar pollinosis were diagnosed based on the patient's clinical history and a positive serum allergen specific IgE antibody test result.

Study Design

This cross-sectional study was performed at Showa University Hospital between September 2014 and December 2014. The medical history was collected from the patients when their asthma was stable. All patients underwent a measurement of body mass index (BMI), lung function test, blood test, and measurement of fractional exhaled nitric oxide (FeNO). The study protocol was reviewed and approved by the Showa University's ethics committee, and written informed consent was obtained from each participant.

Measurement of the Serum Total and Allergen Specific IgE Antibodies

The serum total IgE levels and antigen specific IgE levels were measured with a fluorescent enzyme immunoassay (ImmunoCAP-FEIA, Thermo Fisher Scientific Inc, Uppsala, Sweden), and the data were transformed logarithmically to normalize the distribution. A zero was replaced with a value half of the lowest value observed before log transformation. The specific IgE levels were determined for 16 aeroallergens: *Dermatophagoides pteronyssinus* (HDM), *Cryptomeria japonica* (Japanese cedar), cocksfoot, ragweed, mugwort, *Aspergillus fumigatus*, *Alternaria alternata*, *Candida albicans*, *Cladosporium herbarum*, *Penicillium chrysogenum*, *Mucor racemosus*, *Trichophyton rubrum*, cat dander, dog dander, cockroach, and *Bombyx mori* (moth). Specific IgEs were categorized into 6 groups: (1) HDM, (2) Japanese cedar, (3) various types of pollen (cocksfoot, ragweed, and mugwort), (4) fungi (*Aspergillus*, *Alternaria*, *Candida*, *Cladosporium*, *Penicillium*, *Mucor*, *Trichophyton*), (5) animals (cat dander and dog dander), and (6) insects (cockroach and moth). Sensitization was defined as a specific IgE level greater than 0.10 IU/mL.

Assessments

Spirometry was performed using an AS-302 spirometer (Minato Medical Science Co Ltd, Osaka, Japan) in accordance with the American Thoracic Society/European Respiratory Society guidelines^{16,17} to determine the forced expiratory volume in 1 second (FEV₁), forced vital capacity (FVC), and FEV₁/FVC ratio. Patients underwent this test while standing with their noses clipped. The highest value from 3 technically satisfactory attempts was recorded. The FEV₁ and FVC values are expressed as a percentage of the predicted value.

The FeNO level was measured using a portable device (NIOX MINO; Aerocrine AB, Stockholm, Sweden) at an expiratory flow rate of 50 mL/s for 10 seconds, according to the American Thoracic Society guidelines and the manufacturer's instructions.¹⁸

Asthma control was scored weekly using the GINA guidelines, and it was classified as follows: 0, controlled; 1, partly controlled; or 2, uncontrolled. Assessments of the asthma control status were based on the following variables: (1) daytime symptoms, (2) limitations of activities, (3) nocturnal symptoms and awakening, (4) the need for a reliever or rescue treatment, (5) lung function by peak expiratory flow or FEV₁, and (6) exacerbations. Then the scores were averaged for 4 weeks and reclassified as controlled (≤ 1), partly controlled (between 1 and 2), and uncontrolled (2). Asthma control was also assessed using the validated Japanese version of the Asthma Control Test (ACT). Patients' degree of impairment caused by their asthma during the preceding 4 weeks was subjectively evaluated by 5 questions regarding their activity limitations, shortness of breath, nighttime symptoms, use of a rescue medication, and the patient's overall rating of asthma control.

Statistical Analyses

Results are expressed as mean (SD) for continuous variables. All analyses were performed using JMP pro 12 (SAS Institute Inc, Cary, North Carolina). Values that were not normally distributed were log-transformed to obtain normal distribution for regression analysis. Differences in the continuous variables were analyzed using the Mann-Whitney test, and differences in the categorical variables were analyzed using the Pearson χ^2 test. To assess the association between asthma control and specific IgEs to aeroallergens, logistic regression analyses were performed to estimate the odds ratio (OR). These models were adjusted for age, sex, and the remaining aeroallergens. To examine the association between the serum total log-transformed IgE (log-tIgE) and the specific IgE to aeroallergens, multiple linear regression analyses were performed. $P < .05$ was considered significant for all statistical assessments.

Results

A total of 160 patients with adult asthma participated in this study. Patients' mean (SD) age was 58.9 (15.3) years with a range of 20 to 79 years, and the mean (SD) age at asthma onset was 32.5 (20.9) years. The mean (SD) BMI (calculated as weight in kilograms divided by the square of height in meters) was 22.8 (3.4), and 87 patients (54.4%) were women. Ten patients (6.2%) were current smokers, 46 (28.8%) were ex-smokers, and 104 (65.0%) were never smokers at the time of enrollment. All patients were using inhaled corticosteroids. According to the GINA medication steps, 25.0% of patients were treated by step 1 or 2, 39.4% were treated by step 3, 23.7% were treated by step 4, and 11.9% were treated by step 5. No patients were treated with antifungal therapy. The mean (SD) peripheral eosinophil count was 353.5/ μ L (286.8/ μ L) (5.5% [4.3%]). The mean (SD) tIgE level was 936.8 (2853.1) IU/mL, and the mean (SD) log-tIgE level was 2.34 (0.70). The mean (SD) FVC and FEV₁ were 2.85 (0.94) L (90.2% [16.5%]) and 2.08 (0.83) L (78.7% [20.1%]), respectively. The mean (SD) FeNO level was 52.0 (40.7) ppb, which ranged from 10 to 202 ppb.

The prevalence of sensitization to airborne allergens is summarized in Table 1. The prevalence of sensitization to HDM and Japanese cedar was comparable, and these allergens had the highest levels among the 16 airborne allergens, followed by *Candida* and ragweed. Regarding sex differences, sensitization to airborne allergens, except for the Japanese cedar, was higher in men than in women. In particular, the prevalence of sensitization to HDM, mugwort, *Aspergillus*, *Candida*, *Penicillium*, *Trichophyton*, and cockroach was significantly higher in men than in women. Despite no statistical significance, other fungal allergens, such as *Alternaria*, *Cladosporium*, and *Mucor*, also had a higher prevalence of sensitization in men than in women. The mean log-tIgE level was also higher in men than in women (2.51 [0.68] in men and 2.26 [0.70] in women, $P = .01$). We evaluated the association between the log-tIgE and sensitization to airborne allergens. The sensitization to HDM and *Mucor* was significantly associated with the log-tIgE ($P = .008$ and $P < .001$, respectively).

We evaluated the effect of sensitization to airborne allergens on asthma control. Logistic regression models revealed that fungal sensitization was associated with a significantly increased risk of poor asthma control (OR, 5.07; 95% confidence interval [CI], 1.63–22.43; $P < .004$; Table 2). In contrast, there were no associations between asthma control and sensitization to HDM, Japanese cedar, various types of pollen, furry animals, and insects. Similar

Table 2

Association Between Poor Asthma Control (Partly Controlled and Poorly Controlled Asthma) and Sensitization to Airborne Allergens (Specific IgE Level >0.10 IU/mL)

Allergen	OR (95% CI) ^a	P value
House dust mite	0.82 (0.32–2.26)	.70
Japanese cedar	0.45 (0.18–1.14)	.09
Pollen	0.50 (0.19–1.22)	.13
Fungi	5.07 (1.63–22.43)	.004
Furry animals	1.21 (0.46–3.15)	.69
Insects	0.64 (0.26–1.55)	.32

Abbreviations: CI, confidence interval; OR, odds ratio.

^aAdjusted for age and sex.

results were obtained when 0.35 IU/mL was set as the cutoff point (eTable 1). Next, we assessed the effect of sensitization to individual airborne allergens on asthma control. Sensitization to *Aspergillus* and *Penicillium* was associated with a significantly increased risk of poor asthma control (OR, 6.77; 95% CI, 2.66–18.77; $P < .001$; OR, 8.21; 95% CI, 3.01–24.03; $P < .001$, respectively; Table 3). When 0.35 IU/mL was set as the cutoff point, the OR for having poor asthma control was significantly increased when the patient was sensitized to *Aspergillus*, *Penicillium*, and *Trichophyton*; in contrast, sensitization to the Japanese cedar was negatively associated with having poor asthma control (eTable 2).

Asthma severity is defined by asthma control levels and the treatment status according to the GINA guidelines. Therefore, we evaluated the association between sensitization to individual airborne allergens and the asthma treatment steps. ORs for being treated with step 4 or 5 were significantly increased when the patient was sensitized to *Aspergillus* (OR, 2.20; 95% CI, 1.10–4.45; $P = .02$; Table 4). Despite no statistical significance, sensitization to *Penicillium* also had a high OR of 1.85, followed by *Aspergillus*. These data indicate the association between sensitization to *Aspergillus* and *Penicillium* and an increased asthma severity.

Then we compared data for the patients' demographic characteristics, log-tIgE, and peripheral eosinophil counts between *Penicillium* IgE-positive patients and *Penicillium* IgE-negative patients to elucidate the characteristics of patients with sensitization to *Penicillium* (Table 5). More *Penicillium* IgE-positive patients were men and pet owners compared with *Penicillium* IgE-negative patients ($P < .001$ and $P = .01$, respectively); in addition, *Penicillium* IgE-positive patients had higher log-tIgE levels than *Penicillium* IgE-negative patients ($P < .001$). Moreover, no significant

Table 1
Prevalence of Sensitization to Airborne Allergens

Allergen	No. (%) of patients			P value ^a
	Total (n = 160)	Men (n = 73)	Women (n = 87)	
House dust mite	118 (73.8)	60 (82.2)	58 (66.7)	.03
Japanese cedar	120 (75.0)	54 (74.0)	66 (75.9)	.78
Cocksfoot	57 (35.6)	27 (37.0)	30 (34.5)	.74
Ragweed	73 (45.6)	39 (53.4)	34 (39.1)	.07
Mugwort	53 (33.1)	30 (41.1)	23 (26.4)	.049
<i>Aspergillus fumigatus</i>	54 (33.8)	31 (42.5)	23 (26.4)	.03
<i>Alternaria alternata</i>	35 (21.9)	21 (28.5)	14 (16.1)	.053
<i>Candida albicans</i>	76 (47.5)	41 (56.2)	35 (40.2)	.04
<i>Cladosporium herbarum</i>	27 (16.9)	16 (21.9)	11 (12.6)	.12
<i>Penicillium chrysogenum</i>	35 (21.9)	24 (32.9)	11 (12.6)	.002
<i>Mucor racemosus</i>	38 (23.8)	22 (30.1)	16 (18.4)	.08
<i>Trichophyton rubrum</i>	45 (28.1)	32 (43.8)	13 (14.9)	<.001
Cat dander	62 (38.8)	30 (41.1)	32 (36.8)	.58
Dog dander	62 (38.8)	28 (38.4)	34 (39.1)	.92
Cockroach	68 (42.5)	41 (56.2)	27 (31.0)	.001
Moth	88 (55.0)	45 (61.6)	43 (49.4)	.12

^aThe significant differences between men and women are in boldface ($P < .05$).

Table 3

Association Between Poor Asthma Control (Partly Controlled and Poorly Controlled Asthma) and Sensitization to Individual Airborne Allergen (Specific IgE Level >0.10 IU/mL)

Allergen	OR (95% CI) ^a	P value
House dust mite	0.82 (0.32–2.26)	.70
Japanese cedar	0.45 (0.18–1.14)	.09
Cocksfoot	0.41 (0.13–1.10)	.08
Ragweed	0.44 (0.16–1.10)	.08
Mugwort	0.50 (0.15–1.39)	.19
<i>Aspergillus fumigatus</i>	6.77 (2.66–18.77)	<.001
<i>Alternaria alternata</i>	1.81 (0.63–4.92)	.26
<i>Candida albicans</i>	1.65 (0.69–4.05)	.26
<i>Cladosporium herbarum</i>	1.58 (0.47–4.72)	.44
<i>Penicillium chrysogenum</i>	8.21 (3.01–24.03)	<.001
<i>Mucor racemosus</i>	1.49 (0.53–3.93)	.43
<i>Trichophyton rubrum</i>	2.50 (0.93–6.73)	.07
Cat dander	0.82 (0.29–2.18)	.69
Dog dander	1.03 (0.38–2.69)	.96
Cockroach	0.81 (0.31–1.99)	.64
Moth	0.79 (0.33–1.94)	.62

Abbreviations: CI, confidence interval; OR, odds ratio.

^aAdjusted for age and sex.

Table 4

Association Between High Treatment Steps (Step 4 or 5) and Sensitization to Aeroallergens

Allergen	OR (95% CI) ^a	P value
House dust mite	1.28 (0.60–2.80)	.54
Japanese cedar	0.88 (0.42–1.89)	.74
Cocksfoot	1.11 (0.56–2.17)	.76
Ragweed	1.17 (0.60–2.29)	.64
Mugwort	0.99 (0.48–2.05)	.10
<i>Aspergillus fumigatus</i>	2.20 (1.10–4.45)	.02
<i>Alternaria alternata</i>	0.92 (0.40–2.08)	.86
<i>Candida albicans</i>	1.27 (0.66–2.47)	.47
<i>Cladosporium herbarum</i>	1.35 (0.55–3.28)	.51
<i>Penicillium chrysogenum</i>	1.85 (0.83–4.16)	.13
<i>Mucor racemosus</i>	1.23 (0.56–2.67)	.59
<i>Trichophyton rubrum</i>	1.40 (0.65–3.01)	.39
Cat dander	1.41 (0.68–2.93)	.36
Dog dander	1.37 (0.66–2.85)	.39
Cockroach	1.53 (0.78–3.07)	.22
Moth	0.77 (0.39–1.51)	.45

Abbreviations: CI, confidence interval; OR, odds ratio.

^aAdjusted for age and sex.

differences were observed in terms of the mean age, BMI, asthma duration, smoking status, positivity of allergic rhinitis and pollinosis, and peripheral eosinophils between *Penicillium* IgE-positive patients and *Penicillium* IgE-negative patients. Regarding asthma control, the ACT levels were significantly lower in *Penicillium* IgE-positive patients than in *Penicillium* IgE-negative patients ($P = .023$; Fig 1A). However, the FeNO levels and pulmonary function test results (FVC and FEV₁) were not significantly different between *Penicillium* IgE-positive patients and *Penicillium* IgE-negative patients (Fig 1B and Fig 2A and B).

Finally, we assessed which airborne allergens are involved in sensitization to *Penicillium*. Sensitization to *Penicillium* was associated with sensitization to all airborne allergens, except Japanese cedar, when adjusted by age and sex (Table 6). However, when adjusted by individual airborne allergens in addition to age and sex, sensitizations to *Aspergillus*, *Cladosporium*, and *Trichophyton* were associated with sensitization to *Penicillium* (OR, 7.02; 95% CI, 1.34–43.02; $P = .02$; OR, 31.03; 95% CI, 3.93–413.87; $P < .001$; and OR, 10.26; 95% CI, 2.24–53.37; $P = .003$, respectively; Table 6). Multiple linear regression analysis using raw data also revealed that sensitization to *Aspergillus*, *Cladosporium*, and *Trichophyton* was positively correlated with sensitization to *Penicillium*. (eTable 3). Among 35 *Penicillium*-sensitized patients (21.9%), 30 (18.8%) overlapped with *Aspergillus* and *Penicillium* (Fig 3).

Table 5Demographic Characteristics of Study Participants by Sensitization to *Penicillium chrysogenum*

Characteristic	<i>Penicillium chrysogenum</i> IgE negative (n = 125)	<i>Penicillium chrysogenum</i> IgE positive (n = 35)	P value
Age, y ^a	59.89 (14.65)	55.26 (17.21)	.21
Male sex, No. (%)	49 (39.20)	24 (68.57)	<.001
BMI ^a	23.10 (3.44)	22.50 (3.27)	.36
Asthma duration ^a	26.48 (17.07)	26.53 (19.01)	.90
Smoking, current:ex:never, %	5.6:24.8:69.6	8.6:42.8:48.6	.07
Presence of AR, %	49.60	34.29	.12
Presence of pollinosis, %	50.40	34.29	.09
Pet owner, %	17.60	37.14	.01
Total IgE (log), IU/L ^{a,b}	2.19 (0.60)	2.99 (0.67)	<.001
Peripheral eosinophils, /μL ^a	362.69 (282.50)	320.61 (304.07)	.35

Abbreviations: AR, allergic rhinitis; BMI, body mass index (calculated as weight in kilograms divided by the square of height in meters).

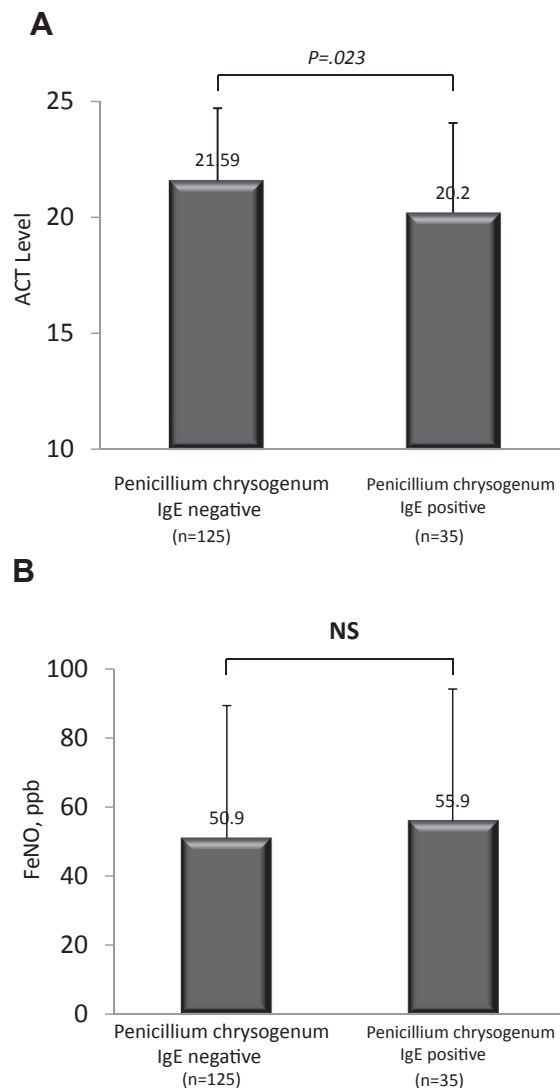
^aData are expressed as mean (SD) unless otherwise indicated.^bTotal IgE levels were the mean of the log-transformed IgE levels.

Figure 1. Mean Asthma Control Test (ACT) levels (A) and fractional exhaled nitric oxide (FeNO) levels (B) in *Penicillium chrysogenum* IgE-positive patients and *Penicillium chrysogenum* IgE-negative patients. The numbers above the bars indicate the mean levels. NS, no significance.

Discussion

In the present study, we found that fungal sensitization is significantly associated with poor asthma control in Japanese adults with asthma. Our study found that positive sensitizations to individual fungi are higher in men than in women, despite no significance in terms of *Alternaria*, *Cladosporium*, and *Mucor*. We also identified that the log-tIgE is higher in men than in women. Similar results were previously found in patients with asthma across all age groups.¹⁹ We previously reported that men were more likely than women to be sensitized to *Staphylococcus aureus* enterotoxins A, which was associated with poor asthma control.²⁰ Intriguingly, there was no sex difference in terms of positive sensitization to various types of pollen and furry animals, except mugwort. It is likely that the proportion of sensitization to fungi associated with poor asthma control is dominant in men. Concerning the association between fungal sensitization and poor asthma control, which had already been adjusted by sex, fungal sensitization was independently associated with poor asthma control. In our study, the family history of atopy, which can influence allergen sensitization, was not considered. Therefore, further

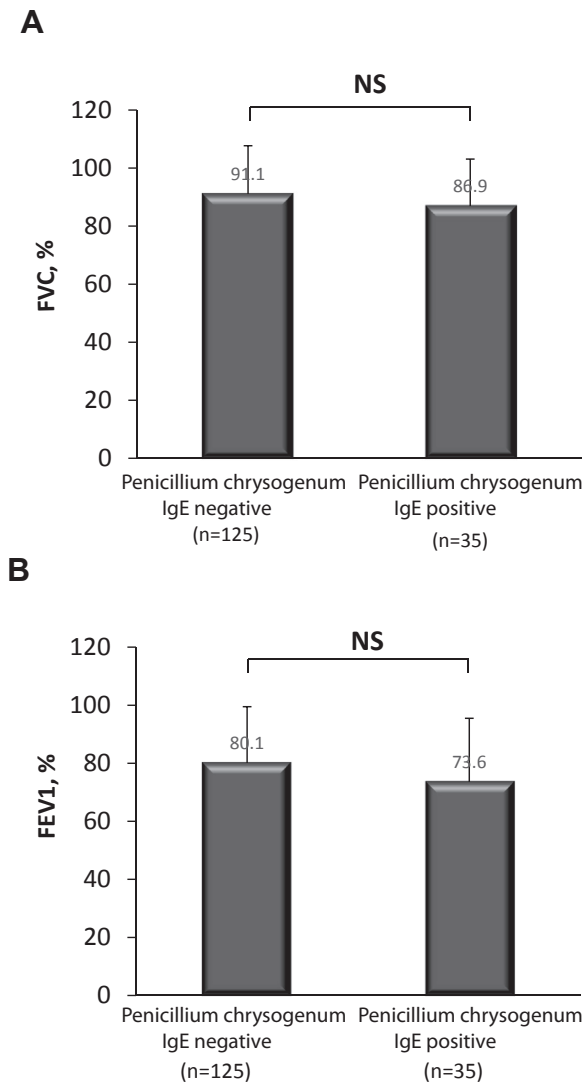


Figure 2. Pulmonary function tests in *Penicillium chrysogenum* IgE-positive patients and *Penicillium chrysogenum* IgE-negative patients. The numbers above the bars indicate the mean levels. A, Forced vital capacity (FVC); B, forced expiratory volume in 1 second (FEV₁). NS, no significance.

studies are needed to determine the influence of family history of atopy on the association between fungal sensitization and poor asthma control.

A previous study conducted by Patelis et al²¹ found that sensitization to fungi and furry animals was associated with an increase in poor asthma control and eosinophilic inflammation, which was identified by the increase in FeNO and peripheral eosinophil count in patients with asthma. To the contrary, there was no association between sensitization to furry animals and poor asthma control in our study. As indicated in Table 5, the prevalence of owning a pet, mostly dogs or cats, was significantly higher, and asthma control was significantly poorer in *Penicillium* IgE-positive patients than in *Penicillium* IgE-negative patients, suggesting that owning pets is associated with fungal sensitization and poor asthma control. A further study with more participants is needed to determine the substantial effects of sensitization to a furry animal on asthma control. In the present study, no association was found between sensitization to various types of pollen and poor asthma control, which is in agreement with that found in previous studies.^{21,22} The size of fungal spores is generally smaller than pollen. Therefore, fungal spores can possibly reach the distal airway area and induce

Table 6

Association Between Sensitization to *Penicillium chrysogenum* and Other Airborne Allergens

Allergen	OR (95% CI) ^a	P value	OR (95% CI) ^b	P value
House dust mite	3.81 (1.19–17.04)	.02	1.34 (0.19–11.02)	.77
Japanese cedar	1.79 (0.69–5.29)	.24	0.93 (0.16–5.30)	.94
Cocksfoot	3.19 (1.44–7.30)	.004	0.40 (0.03–3.41)	.41
Ragweed	2.90 (1.28–6.90)	.01	0.54 (0.06–4.06)	.54
Mugwort	3.89 (1.67–9.33)	.002	5.12 (0.89–31.24)	.07
<i>Aspergillus fumigatus</i>	24.44 (9.02–79.97)	<.001	7.02 (1.34–43.02)	.02
<i>Alternaria alternata</i>	18.12 (7.01–51.54)	<.001	1.57 (0.19–13.20)	.67
<i>Candida albicans</i>	4.61 (1.96–11.81)	<.001	1.89 (0.38–9.64)	.43
<i>Cladosporium herbarum</i>	39.10 (12.35–151.71)	<.001	31.03 (3.93–413.87)	<.001
<i>Mucor racemosus</i>	7.48 (3.21–18.15)	<.001	0.14 (0.01–1.47)	.11
<i>Trichophyton rubrum</i>	21.84 (8.38–64.22)	<.001	10.26 (2.24–53.37)	.003
Cat dander	3.08 (1.31–7.49)	.01	0.27 (0.03–1.99)	.20
Dog dander	4.28 (1.76–11.01)	.001	2.02 (0.21–17.35)	.53
Cockroach	4.59 (1.97–11.49)	<.001	0.57 (0.06–5.74)	.63
Moth	3.11 (1.30–8.15)	.01	1.72 (0.20–13.92)	.61

Abbreviations: CI, confidence interval; OR, odds ratio.

^aAdjusted for age and sex.

^bAdjusted for age, sex, and the rest of the allergen.

chronic airway inflammation of the lung tissue.¹³ However, there is increasing evidence that pollen fragments (subpollen particles) can be inhaled into the deeper parts of the lung.²³ A previous study from Japan found poor asthma control in patients with comorbid rhinitis compared with patients without rhinitis only during the pollen season, usually from March to April.²⁴ This study suggests that the season and pollen species should be considered when evaluating the influence of sensitization to pollen on asthma control.

The present study found that sensitization to *Aspergillus* and *Penicillium* was positively associated with poor asthma control. Although sensitizations to other fungi tended to be associated with poor asthma control, there were no significant differences. Few reports have compared immune responses to common inhalant fungi in relation to poor asthma control together with other airborne allergens. Moreover, the association between sensitization to *Penicillium* and poor asthma control in patients with adult asthma has not been fully elucidated. Despite no significance, the OR for the association between step 4 or 5 treatment and

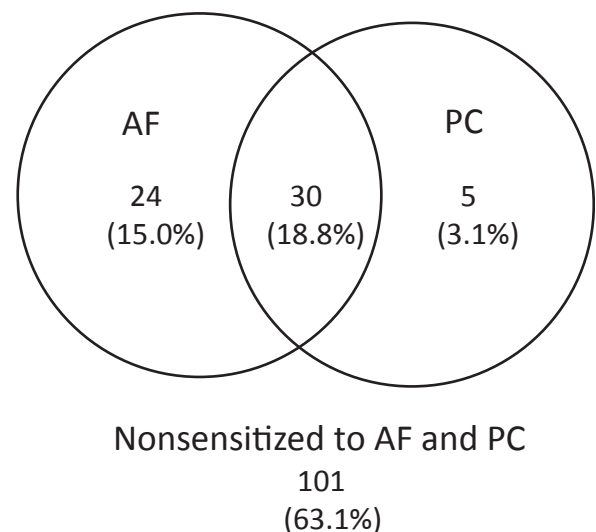


Figure 3. Overlapping sensitization to *Aspergillus fumigatus* (AF) and *Penicillium chrysogenum* (PC). The circles show the numbers and percentage of individuals positive for AF or PC. The area within the diagram but outside the circles represents the proportions of nonsensitized to AF and PC.

sensitization to *Penicillium* was 1.85, which was the second only to *Aspergillus*. In addition, no participants had poor adherence to treatment in this study. These data suggest that the association between sensitization to *Penicillium* and poor asthma control is not attributable to the insufficiency of asthma treatment or poor adherence. *Penicillium* is the most commonly isolated fungus in indoor dust samples,^{25,26} and it belongs to the Trichocomaceae family, which is the same family of *Aspergillus*.²⁷ In our study, we found a positive association between sensitization to *Penicillium* and *Aspergillus*, even after adjusting for the rest of the allergens. *Penicillium* ranges in diameter from 1 to 5 μm , with a mean size of 3.5 μm , which is similar to that of *Aspergillus*. This relatively small size and round shape allow spores of this organism to penetrate the lower airways when inhaled.²⁸ Major IgE-binding components of *P. chrysogenum* are alkaline serine protease allergens (Pen ch 13) and vacuolar serine protease allergens (Pen ch 18). These proteases are also major IgE-binding components of *Aspergillus* (Asp f 13 and Asp s 18). Because of the high sequence similarity, the cross-reactive action between *Penicillium* and *Aspergillus* may occur regularly when the IgE test or skin prick test (SPT) is performed in clinical practice. It would be difficult to evaluate the influence of sensitization to *Aspergillus* on the association between sensitization to *Penicillium* and poor asthma control.

The current study contains several limitations that need to be acknowledged. First, sensitizations to airborne allergens were assessed by only measuring allergen specific IgE not by the SPT. The SPT and measurement of specific serum IgE are the most common methods for diagnosing sensitization to common inhaled allergens. The SPT is more sensitive but less specific than the serum IgE tests to assess sensitizations to airborne allergens. O'Driscoll et al.²⁹ found that concordance rates between the SPT and specific serum IgE tests for sensitization to fungi and *Penicillium* were 77% and 29%, respectively, in 121 patients with severe asthma. They also found that the sensitivity of fungal sensitization was slightly higher with the specific IgE test than with the SPT. Therefore, a simultaneous assessment of fungal sensitization by the SPT and specific serum IgE test would further elucidate the influence of fungal sensitization on asthma control. Second, the definition of specific serum IgE positive or negative is not standardized. Currently, the technical advances of ImmunoCAP-FEIA have increased the sensitivity of specific IgE. We previously found that patients whose IgE level against Japanese cedar was less than 0.35 IU/mL, which was classified as a negative result, had cedar pollinosis, mostly induced by Japanese cedar, and their IgE level against Japanese cedar increased above 0.35 IU/mL after treatment with omalizumab.³⁰ Thus, we defined the specific IgE level of 0.10 IU/mL or higher as positive in this study. Even when 0.35 IU/mL was set as the cutoff point, sensitization to *Aspergillus* and *Penicillium* was positively associated with poor asthma control. Third, no differences were found in the FeNO levels and pulmonary function tests, including FVC and FEV₁ percentages, between *Penicillium* IgE-positive patients and *Penicillium* IgE-negative patients. To confirm this finding, further studies with a larger sample size are needed.

In conclusion, fungal sensitization, particularly to *Aspergillus* and *Penicillium*, is positively associated with poor asthma control in Japanese adults with asthma. We also found that more men than women are sensitized to fungi, including *Aspergillus* and *Penicillium*. Therefore, the immune response to *Aspergillus* and *Penicillium* may substantially contribute to poor asthma control, particularly in men.

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Supplementary Data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.anai.2016.06.001>.

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eTable 1

Association Between Poor Asthma Control (Partly Controlled and Poorly Controlled Asthma) and Sensitization to Airborne Allergens (Specific IgE Level >0.35 IU/mL)

Allergen	OR (95% CI) ^a	P value
House dust mite	0.85 (0.33–2.21)	.74
Japanese cedar	0.39 (0.16–0.94)	.04
Pollen	0.37 (0.08–1.19)	.10
Fungi	2.62 (1.08–6.66)	.03
Furry animals	1.43 (0.52–3.75)	.48
Insects	0.64 (0.24–1.60)	.34

Abbreviations: CI, confidence interval; OR, odds ratio.

^aAdjusted for age and sex.

eTable 3

Multiple Linear Regression Analysis of *Penicillium chrysogenum* Specific IgE in Patients with Asthma

Specific IgE	Coefficient (95% CI)	β standardized coefficient	P value
House dust mite	−0.029 (−0.10 to 0.05)	−0.046	.45
Japanese cedar	0.032 (−0.04 to 0.11)	0.050	.40
Cocksfoot	−0.059 (−0.18 to 0.06)	−0.069	.34
Ragweed	−0.110 (−0.27 to 0.05)	−0.116	.18
Mugwort	0.114 (−0.02 to 0.25)	0.118	.10
<i>Aspergillus fumigatus</i>	0.270 (0.15 to 0.39)	0.321	<.001
<i>Alternaria alternata</i>	−0.086 (−0.57 to 0.39)	−0.105	.72
<i>Candida albicans</i>	0.159 (−0.32 to 0.63)	0.193	.51
<i>Cladosporium herbarum</i>	0.519 (0.32 to 0.72)	0.432	<.001
<i>Mucor racemosus</i>	−0.139 (−0.31 to 0.03)	−0.124	.11
<i>Trichophyton rubrum</i>	0.226 (0.11 to 0.34)	0.258	<.001
Cat dander	−0.027 (−0.15 to 0.10)	−0.038	.68
Dog dander	−0.033 (−0.18 to 0.11)	−0.044	.66
Cockroach	0.050 (−0.10 to 0.20)	0.056	.498
Moth	0.063 (−0.05 to 0.17)	0.081	.26

Abbreviation: CI, confidence interval.

eTable 2

Association Between Poor Asthma Control (Partly Controlled and Poorly Controlled Asthma) and Sensitization to Individual Airborne Allergens (Specific IgE Level >0.35 IU/mL)

Allergen	OR (95% CI) ^a	P value
House dust mite	0.85 (0.33–2.21)	.74
Japanese cedar	0.39 (0.16–0.94)	.04
Cocksfoot	0.41 (0.06–1.53)	.21
Ragweed	0.24 (0.01–1.24)	.10
Mugwort	0.60 (0.09–2.43)	.51
<i>Aspergillus fumigatus</i>	3.32 (1.23–8.71)	.02
<i>Alternaria alternata</i>	0.95 (0.14–4.04)	.94
<i>Candida albicans</i>	1.21 (0.43–3.16)	.70
<i>Cladosporium herbarum</i>	2.81 (0.54–12.25)	.20
<i>Penicillium chrysogenum</i>	3.26 (1.01–9.99)	.049
<i>Mucor racemosus</i>	1.05 (0.15–4.41)	.95
<i>Trichophyton rubrum</i>	4.95 (1.67–15.62)	.004
Cat dander	1.12 (0.38–3.12)	.83
Dog dander	1.77 (0.64–4.66)	.27
Cockroach	0.64 (0.14–2.13)	.49
Moth	0.68 (0.25–1.72)	.42

Abbreviations: CI, confidence interval; OR, odds ratio.

^aAdjusted for age and sex.