

Specific Detection of *Bjerkandera adusta* by Polymerase Chain Reaction and Its Incidence in Fungus-Associated Chronic Cough

Mariko Yamaura · Kazuo Satoh ·
Takashi Yamazaki · Haruhiko Ogawa ·
Koichi Makimura

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Abstract Chronic cough is a common symptom at outpatient care. An uncontrollable cough with difficulty in treatment is called chronic idiopathic cough. Recent reports have demonstrated that the presence of basidiomycetous fungi in sputum is an important clinical finding that assists in clarifying the cause of chronic cough in some cases. Research has suggested that *Bjerkandera adusta* is related to fungus-associated chronic cough (FACC). FACC is defined as a chronic cough associated with basidiomycetous fungi found in induced sputum and can be treated with antifungal medication. *B. adusta* is one of the basidiomycetous fungi that exist in cosmopolitan environments. The aim of this study was to develop a *B. adusta* detection method using polymerase chain

reaction (PCR) with a specific primer set and to research the incidence of *B. adusta* in FACC. The new method successfully detected *B. adusta* from FACC patients. The incidence of *B. adusta* in FACC was 42.86 %. Antifungal drugs were effective in most cases. Significant differences in treatment duration between *B. adusta* patients and non-*B. adusta* patients were observed. It is therefore suggested that the presence of *B. adusta* may be one of the allergic intractable factors of chronic cough. This finding may provide identifiable differences in clinical manifestations between *B. adusta* and non-*B. adusta* in FACC and lead to possible differing remedies to treat the two forms. PCR can specifically detect *B. adusta* from patients suffering from chronic cough and provides a new diagnosis for FACC associated with *B. adusta*.

M. Yamaura · K. Satoh · T. Yamazaki ·
K. Makimura (✉)
Laboratory of Space and Environmental Medicine,
Graduate School of Medicine, Teikyo University,
Tokyo 173-8605, Japan
e-mail: makimura@med.teikyo-u.ac.jp

K. Satoh · T. Yamazaki · K. Makimura
Institute of Medical Mycology and Genome Research
Center, Teikyo University, Tokyo 192-0395, Japan

K. Satoh · T. Yamazaki · H. Ogawa · K. Makimura
General Medical Education Center, Teikyo University,
Tokyo 173-8605, Japan

H. Ogawa
Division of Pulmonary Medicine, Ishikawa-ken Saiseikai
Kanazawa Hospital, Kanazawa 920-0353, Japan

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Introduction

Chronic cough is a common symptom prompting people to see a doctor and is the fifth most typical complaint seen at outpatient care in the world [1]. It can produce chest pain, ruin sleep and lead to poor concentration. Furthermore, it can result in an impaired quality of life and sometimes causes

psychological problem [2]. Even if guidelines on diagnosis and management are followed [3–5], effective management of patients with chronic cough is difficult, and failure to obtain a treatment response may lead to misdiagnosis. Studies have reported that causes of chronic cough can remain unexplained in 33–46 % of cases [6, 7]. An uncontrollable cough is termed a chronic idiopathic cough [8–10].

It is necessary to investigate the causes of chronic idiopathic cough and investigate treatments to reduce the severity and frequency of the illness to provide patients with a better quality of life. Our previous studies revealed that the presence of basidiomycetous (BM) fungi in induced sputum is an important clinical finding, which assists in clarifying the cause of chronic cough in some cases [11, 12]. Clinical responses to antiallergic drugs have not shown good results, but antifungal drug therapies have shown efficacy in some cases [13, 14].

Ogawa et al. [15] proposed a new clinical concept called fungus-associated chronic cough (FACC) in 2009. FACC is defined as: (1) chief complaint is chronic cough; (2) environmental fungi, especially BM fungi, are cultured from sputum; and (3) antifungal drugs are effective. A specific and sensitive method to rapidly identify and detect BM fungi species that are associated with FACC in sputum is necessary. However, trouble with morphological identification makes accurate diagnosis difficult. Recently, molecular biology techniques, especially polymerase chain reaction (PCR), have provided an alternative approach for the identification and detection of pathogenic fungi [16]. Such simple, quick and specific diagnostic assays are essential for studying allergenic BM fungi. As noted, the importance of the relationship between *Bjerkandera adusta*, in particular, which is a BM fungi, and FACC has been revealed [17, 18].

The aim of the current study was to develop a *B. adusta* detection method using PCR with specific primer sets and to investigate the incidence of *B. adusta* in FACC.

Materials and Methods

Fungal Strains and Culture

The fungal standard strains used in this study (7 species, 12 strains) are shown in Table 1.

Table 1 Standard strains tested in the study

Species	Strains
<i>Bjerkandera adusta</i>	NBRC106826, NBRC 5307, NBRC 104974, NBRC 4983
<i>Aspergillus fumigatus</i>	TIMM 0108, TIMM 2920
<i>Aspergillus niger</i>	JCM 10254
<i>Cryptococcus neoformans</i>	ATCC 90113
<i>Candida albicans</i>	ATCC 90108, ATCC 10231
<i>Candida dublinensis</i>	CBS 7987
<i>Trichosporon asahii</i>	TIMM 1318

NBRC Biological Research Center, National Institute of Technology and Evaluation (Japan), *TIMM* The Teikyo University Institute of Medical Mycology (Japan), *JCM* Japan Collection of Microorganisms (Japan), *ATCC* American Type Culture Collection (United States), *CBS* The Convention of Biological Diversity (the Netherlands)

The clinical specimens (sputum, pharynx swab and bronchoalveolar lavage) were obtained from each patient whose chief complaint was chronic cough between 2005 and 2012 at Ishikawa-ken Saiseikai Kanazawa Hospital, Kanazawa, Ishikawa, Japan. Informed consent was obtained from all patients. All the specimens were cultured at 28 °C on Sabouraud dextrose agar (1 % peptone, 2 % glucose, 1.5 % agar) spread with 30 µL of micafungin solution (30-µg 90-mm plate⁻¹) [19] for 5 days at the Laboratory of Space and Environmental Medicine, Graduate School of Medicine, Teikyo University, Tokyo, Japan. DNAs from white aerial mycelia grown on the agar were isolated and subjected to PCR analysis.

Oligonucleotide Primers

The rRNA gene sequences of the fungal species in Table 1 were multiple aligned using Genetyx Ver. 10 software (Genetyx Co. Ltd., Tokyo, Japan). The species-specific primer set F3 (5'-ACCTTGCGCTCC TTGGTAT-3')/B3 (5'-CTCCACAGCAACGCAGA T-3') for *B. adusta* was designed between the 18S rRNA gene and the 28S rRNA (D1/D2) gene (DDBT/EMBL/GenBank Accession No. AB733157). This primer set was designed to amplify a single *B. adusta* DNA product of 194 bp located between 5.8S and ITS2 on the rRNA gene base sequence.

The primer set 18SF1 (5'-AGGTTTCCGTAGGTG AACCT-3')/58SR1 (5'-TTCGCTGCGTTCTTCATC GA-3') developed by Makimura et al. [20] was used for universal detection of DNA derived from fungi. As

a positive control, artificial *B. adusta* DNA was synthesized based on DDBT/EMBL/GenBank Accession No. AB733157 by Funakoshi Company (Tokyo, Japan) and cloned into a pEX-A vector.

DNA Extraction

Fungal DNA was extracted using phenol–chloroform extraction [20] and stored at -30°C until PCR analysis.

PCR Conditions

Polymerase chain reaction amplification was performed in 50 μL reaction volume containing 5–100 ng of DNA template, 5 μL of $10 \times \text{Ex Taq}$ buffer (Takara, Shiga, Japan), 4 μL of dNTPs (Takara), 10 pmol of each primer, 1 μL of *Ex Taq* DNA polymerase (Takara) and distilled water. Amplification was carried out using a Takara Bio PCR Thermal Cycler Dice® (Takara) under the following conditions: initial 94°C for 4 min, followed by 25 cycles at 94°C for 60 s, 60°C for 15 s, 72°C for 15 s and a final extension at 72°C for 10 min. Amplified PCR products were separated by electrophoresis using 1.0 % agarose gel in $1 \times \text{TAE}$ buffer. PCR products were stained with ethidium bromide and visualized under UV light.

DNA Sequencing

PCR products were purified using a QIAquick® PCR Purification Kit (Qiagen, Valencia, CA, USA) and sequenced using a BigDye® Terminator Sequencing Kit (Applied Biosystems, Carlsbad, CA, USA) and an ABI PRISM® 310 Genetic Analyzer (Applied Biosystems).

Results

Specificity

The designed specific primer set F3/B3 for *B. adusta* had no matches to other fungal species *in silico* using Primer-BLAST (<http://www.ncbi.nlm.nih.gov/tools/primer-blast/>). All standard strains of *B. adusta* were successfully amplified with the primer set F3/B3. No

other DNA was amplified from the other species in Table 1 (Fig. 1).

Sensitivity

Polymerase chain reaction sensitivity was evaluated from 2 ng to 0.2 fg of artificial *B. adusta* DNA. The lowest detection limit was 200 fg (Fig. 2).

Incidence of *B. adusta* in Clinical Isolated Samples from FACC Patients

White aerial mycelia, which are characteristic of BM fungi, were observed in 28 samples (24 sputa samples, 3 pharynx swab samples and 1 bronchoalveolar lavage sample). Twelve DNA samples (10 sputa samples, 1 bronchoalveolar lavage sample and 1 pharynx swab sample) out of the 28 samples were amplified using primer set F3/B3 (42.86 %). Subsequently, all 12 samples were identified as *B. adusta* (100 %) by DNA sequencing and BLAST analysis (DDBT/EMBL/GenBank Accession No. AB096738.1).

PCR amplification products obtained using fungal universal primer set 18SF1/58SR1 were detected in all 28 samples and identified as BM fungi, and 12 out of 28 samples were identified as *B. adusta* by DNA sequencing and BLAST analysis (DDBT/EMBL/GenBank Accession No. AB096738.1). Detection results of all samples are shown in Table 2.

Differences in treatment duration between *B. adusta* patients and non-*B. adusta* patients

Patients whose sputum samples were identified as *B. adusta* required longer treatment than non-*B. adusta* patients (Table 2). A significant difference in treatment duration between *B. adusta* patients [mean (weeks) $\pm$ SD, 5.67 ± 2.67] and non-*B. adusta* patients [mean (weeks) $\pm$ SD, 1.63 ± 0.70] was observed using an independent sample student's *t* test [$t(26) = -3.43$; $p < 0.05$].

Discussion

Fungi exist naturally in the environment and on and in the human body. Fungi do not usually cause disease in humans unless the host is compromised in some way [21]. They are, however, one of the allergens (for

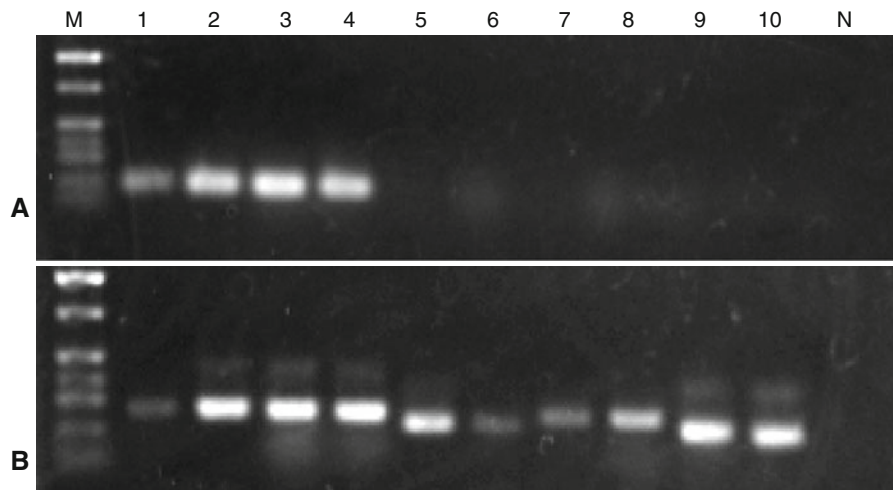


Fig. 1 Specificity of PCR using the F3/B3 primer set. Amplification of DNA from various fungi species was performed using F3/B3 primer set (**a**) and 18SF1/58SR1 primer set (**b**). Lanes: M, DNA marker (50 bp ladder size standard); N, non-template control; 1, amplification of 2 pg of artificial *B.*

adusta DNA; 2, DNA amplification of standard strain of *B. adusta* (NBRC 5307); 3–10, amplification of genomic DNA of (3, 4) *B. adusta*, (5) *Schizophyllum commune*, (6) *Cryptococcus neoformans*, (7) *Aspergillus fumigatus*, (8) *Aspergillus niger*, (9) *Candida albicans*, (10) *Tricosporon asahii*

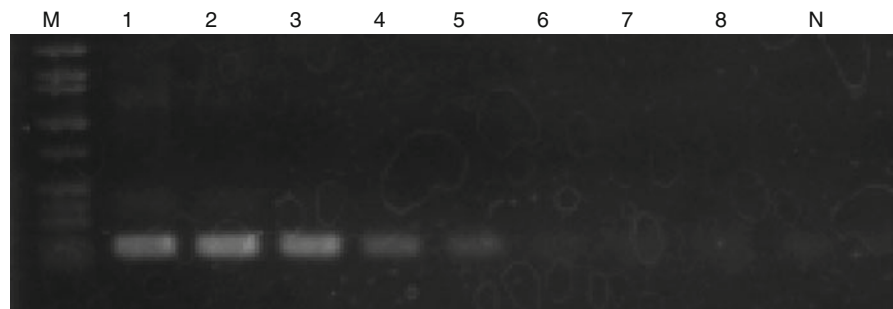


Fig. 2 Sensitivity of specific detection method for *B. adusta*. Lanes: M, DNA marker (50 bp ladder size standard); N, non-template control; 1–8, amplification of artificial DNA of *B.*

adusta. Concentrations: (1) 2 ng, (2) 200 pg, (3) 20 pg, (4) 2 pg, (5) 200 fg, (6) 20 fg, (7) 2 fg, (8) 0.2 fg

example, pollen and mites) that cause bronchial asthma, allergic rhinitis, atopic dermatitis, hypersensitivity pneumonia and allergic bronchopulmonary aspergillosis [22–26]. A large variety of fungi can be antigenic and cause allergic disease independently from their pathogenicity or infectiousness. Studies have reported on exposure to fungi in the home and work environments, and have suggested that identification of environmental fungi as possible causative allergens may be an essential key for the accurate diagnosis of an allergy to a specific fungal species [27–29]. For example, a 28-year-old office worker was reported to suffer from chronic cough. Her symptoms

were exacerbated around midnight, especially during week days but reduced on the weekend [30]. Culture results from her induced sputum identified BM fungi. BM fungi were also cultured from the floor of her office at work but not from her bedroom. Her uncontrollable cough did not show any improvement until an air cleaner was installed. It is not uncommon for those cases that occur around the workplace to be misdiagnosed as psychogenetic cough. In 1952, Gregory and Hirst [31] reported that BM fungi can cause allergy as an aeroallergen. BM fungi can also cause allergic fungal respiratory disease [32–36], yet the mechanism by which this is achieved has not been

Table 2 Detected fungal species and treatment for 28 FACC patients

Case	Sample	Species	Treatment	Duration (wk)	Outcome
1	Sp	<i>B. adusta</i>	ITZ	10	Improvement
2	Sp	<i>B. adusta</i>	ITZ	10	Remission
3	Sp	<i>B. adusta</i>	ITZ	8	Remission
4	Sp	<i>Phlebiopsis gigantea</i>	ITZ	2	Remission
5	Sp	<i>Termitomyces</i> sp.	ITZ	2	Improvement
6	Sp	<i>B. adusta</i>	ITZ	8	Remission
7	Sp	<i>B. adusta</i>	None	–	Remission
8	P	<i>Rigidoporus</i> sp.	ITZ	1	Remission
9	P	<i>B. adusta</i>	ITZ	4	Improvement
10	Sp	<i>B. adusta</i>	ITZ	4	Remission
11	Sp	<i>B. adusta</i>	ITZ	8	Improvement
12	Sp	<i>Termitomyces albuminosus</i>	ITZ	2	Remission
13	Sp	<i>Irpex lacteus</i> , <i>Ceriporiposis subvermispora</i>	ITZ	2	Remission
14	Sp	<i>B. adusta</i>	ITZ	2	Remission
15	Sp	<i>B. adusta</i>	ITZ	6	Remission
16	P	<i>Trametes elegans</i>	ITZ	1	Remission
17	Sp	<i>Ceriporia lacerata</i>	ITZ	1	Remission
18	Sp	<i>Aurantiopileus mayanensis</i>	ITZ	1	Remission
19	Sp	<i>Ceriporia lacerata</i>	ITZ	1	Remission
20	Sp	<i>Polyporus arcularius</i>	ITZ	5	Remission
21	Sp	Basidiomycota	ITZ	1	Remission
22	Sp	<i>Irpex lacteus</i> , <i>Phanerochaete</i> sp.	ITZ	1	Remission
23	Sp	<i>Ceriporiposis</i> sp.	ITZ	1	Remission
24	BAL	<i>B. adusta</i>	ITZ	4	Remission
25	Sp	<i>Phanerochaete sordida</i>	ITZ	2	Remission
26	Sp	<i>B. adusta</i>	ITZ	4	Remission
27	Sp	<i>Ceriporiposis</i> sp.	ITZ	2	Remission
28	Sp	<i>Phlebia tremellosa</i>	ITZ	1	Remission

ITZ 50 mg/day of itraconazole, Sp Sputum, P Pharynx swab, BAL Bronchoalveolar lavage, wk week(s), Remission recurrence not observed without ITZ, Improvement recurrence partly observed without ITZ but symptoms improved

fully elucidated. Etiological aspects of BM fungi are not well understood.

Bjerkandera adusta (smoky bracket fungus in English, *Yakeirotake* in Japanese) is a widely distributed wood decay fungus that occurs around the world. *B. adusta* usually grows during autumn in moist conditions; however, recent findings [37] have demonstrated that filamentous *B. adusta* can grow in temperatures between 4 and 37 °C and produces asexual spores called arthroconidia. It has been hypothesized that arthroconidia somehow colonize the human pharynx, proliferate in the warm human body and produce an allergen that causes chronic cough regardless of the season.

Antifungal therapy greatly improved symptoms of patients with FACC in previous studies [13, 14, 16]. It is therefore possible that antifungal treatment may eliminate colonizing BM fungi and prevent them from producing the antigen that causes chronic cough.

In the current study, primer set F3/B3 was developed specifically to detect *B. adusta* associated with FACC. The specificity of the *B. adusta* detection method using primer set F3/B3 was confirmed by PCR examination with genomic DNA from various fungi, including 28 cases of FACC. In the result, 42.86 % patients whose sputum was BM fungi positive were diagnosed with FACC due to *B. adusta*. Antifungal drug therapies were effective at eliminating symptoms

in most cases. Patients with sputum samples that were identified as *B. adusta* required longer treatment than non-*B. adusta* patients. A significant difference in treatment duration between *B. adusta* patients and non-*B. adusta* patients was observed in the study. It is therefore suggested that the presence of *B. adusta* may be one of the allergic intractable factors of chronic cough. This finding may provide identifiable differences in clinical manifestations between *B. adusta* and non-*B. adusta* in FACC and lead to possible differing remedies to treat the two forms.

Further research has been carried out to examine the efficacy of antifungal therapies as a new treatment [14]. The new method using specific primer set F3/B3 can detect *B. adusta* from patients suffering from chronic cough and provides a new diagnosis for FACC associated with *B. adusta*. The next challenge is underway to evaluate the potential clinical utility of a rapid quantitative real-time PCR assay using sputum samples directly for *B. adusta* in FACC patients.

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Conflict of interest The authors have no conflicts of interest to declare.

References

1. Braman SS, Corrao WM. Chronic cough Diagnosis and treatment. *Prim Care*. 1985;12(2):217–25.
2. French CL, Irwin RS, Curley FJ, Krikorian CJ. Impact of chronic cough on quality of life. *Arch Intern Med*. 1998;158(15):1657–61.
3. Morice AH, Fontana GA, Sovijarvi AR, Pistolesi M, Chung KF, Widdicombe J, et al. The diagnosis and management of chronic cough. *Eur Respir J*. 2004;24(3):481–92.
4. Irwin RS, Baumann MH, Bolser DC, Boulet LP, Braman SS, Brightling CE, et al. American College of Chest Physicians (ACCP). Diagnosis and management of cough executive summary: ACCP evidence-based clinical practice guidelines. *Chest*. 2006;29(1 Suppl):1S–23S.
5. Kohno S, Ishida T, Uchida Y, Kishimoto H, Sasaki H, Shioya T, et al. The Japanese respiratory society guidelines for management of cough. *Respirology*. 2006;11(Suppl 4):S135–86.
6. Chung KF, Pavord ID. Prevalence, pathogenesis and causes of chronic cough. *Lancet*. 2008;371(9621):1364–74.
7. Pratter MR. Unexplained (idiopathic) cough. ACCP evidence-based clinical practice guide lines. *Chest*. 2006; 129(1Suppl):220S–1S.
8. MacGarvey LPA, Ing AJ. Idiopathic cough, prevalence and underlying mechanisms. *Pulm Pharmacol Ther*. 2004;17(6): 435–9.
9. Haque RA, Usmani OS, Barnes PJ. Chronic idiopathic cough: a discrete clinical entity? *Chest*. 2005;127(5):1710–3.
10. MacGarvey LP. Does idiopathic cough exist? *Lung*. 2008;186(Suppl. 1):S78–81.
11. Ogawa H, Fujimura M. Fungal culture from pharyngeal swab and seasonal influence in patients with chronic non-productive cough. *Nihon Kokyuki Gakkai Zasshi*. 2006;44(3):168–72.
12. Ogawa H, Fujimura M, Takeuchi Y, Makimura K. The importance of basidiomycetous fungi cultured from the sputum of chronic idiopathic cough: a study to determine the existence of recognizable clinical patterns to distinguish CIC from non-CIC. *Respir Med*. 2009;103(10):1492–7.
13. Ogawa H, Fujimura M, Tofuku Y. Treatment of atopic cough caused by Basidiomycetes antigen with low-dose itraconazol. *Lung*. 2004;182(5):279–84.
14. Ogawa H, Fujimura M, Takeuchi Y, Makimura K. Clinical experience with low-dose itraconazole in chronic idiopathic cough. *Cough*. 2013;9(1):1.
15. Ogawa H, Fujimura M, Takeuchi Y, Makimura K. Efficacy of itraconazole in the treatment of patients with chronic cough whose sputa yield basidiomycetous fungi-fungus-associated chronic cough (FACC). *J Asthma*. 2009;46(4):407–12.
16. Niessen L. PCR-based diagnosis and quantification of mycotoxin producing fungi. *Int J Food Microbiol*. 2001;119(1-2):38–46.
17. Ogawa H, Fujimura M, Takeuchi Y, Makimura K, Satoh K. Sensitization to *Bjerkandera adusta* enhances severity of cough symptom in patients with fungus-associated chronic cough (FACC). *Med Mycol J*. 2011;52(3):205–12.
18. Ogawa H, Fujimura M, Takeuchi Y, Makimura K. Is *Bjerkandera adusta* important to fungus-associated chronic cough as an allergen? Eight cases' reports. *J Asthma*. 2009;46(8):849–55.
19. Satoh K, Makimura K. *Sporobolomyces koalae* sp. nov., a basidiomycetous yeast isolated from nasal smears of Queensland koalas kept in a Japanese zoological park. *Int J Syst Evol Microbiol*. 2008;58(Pt 12):2983–6.
20. Makimura K, Mochizuki T, Hasegawa A, Uchida K, Saito H, Yamaguchi H. Phylogenetic classification of *Trichophyton mentagrophytes* complex strains based on DNA sequences nuclear ribosomal internal transcribed spacer 1 regions. *J Clin Microbiol*. 1998;36(9):2629–33.
21. Hart PO, Russell E Jr, Remington JS. The compromised host and infection. II. Deep fungal infection. *J Infect Dis*. 1969;120:169–91.
22. Zureik M, Neukirch C, Leynaert B, Liard R, Bousquet J, Neukirch F. Sensitisation to airborne moulds and severity of asthma: cross sectional study from European Community respiratory health survey. *BMJ*. 2002;325(7361):411–4.
23. Li DW, Kendrick D. Indoor aeromycota in relation to residential characteristics and allergic symptoms. *Mycopathologia*. 1995;131(3):149–57.
24. Gaitanis G, Magiatis P, Hantschke M, Bassukas ID, Velegriaki A. The *Malassezia* genus in skin and systemic diseases. *Clin Microbiol Rev*. 2012;25(1):106–41.
25. Kawai T, Tamura M, Murao M. Summer-type hypersensitivity pneumonitis. A unique disease in Japan. *Chest*. 1984;85(3):311–7.

26. Hinson KF, Moon AJ, Plummer NS. Broncho-pulmonary aspergillosis; a review and a report of eight new cases. *Thorax*. 1952;7(4):317–33.
27. O'Conner GT, Walter M, Mitchell H, Kattan M, Morgan WJ, Gruchalla RS, et al. Airborne fungi in the homes of children with asthma in low-income urban communities: the inner-city asthma study. *J Allergy Clin Immunol*. 2004; 114(3):599–606.
28. Gent JF, Ren P, Belanger K, Triche E, Bracken MB, Holford TR, Leaderer BP. Levels of household mold associated with respiratory symptoms in the first year of life in a cohort at risk for asthma. *Environ Health Perspect*. 2002;110(12):A781–6.
29. Lee T, Grinshpun SA, Martuzevicius D, Adhikari A, Crawford CM, Luo J, et al. Relationship between indoor and outdoor bio-aerosols collected with a button inhalable aerosol sampler in urban homes. *Indoor Air*. 2006;16(1):37–47.
30. Ogawa H, Fujimura M. Atopic cough caused by basidiomycetes antigen; The importance as a fungal aeroallergen in occupational environment. *Arerugi*. 2004;53:430–4.
31. Gregory PH, Hirst JM. Possible role of basidiospores as airborne allergens. *Nature*. 1952;170(4323):414.
32. Ogawa H, Fujimura M, Takeuchi Y, Makimura K. Two cases of *Schizophyllum* asthma: is this a new clinical entity or a precursor of ABPM? *Pulm Pharmacol Ther*. 2011;24(5): 559–62.
33. Ogawa H, Fujimura M, Takeuchi Y, Makimura K. A case of sinobronchial allergic mycosis; possibility of basidiomycetous fungi as a causative antigen. *Intern Med*. 2011; 50(1):59–62.
34. Ogawa H, Fujimura M, Takeuchi Y, Makimura K. Impact of *Schizophyllum* sensitization on decline of lung function in asthma. *J Asthma*. 2013;. doi:[10.3109/02770903.2013.803573](https://doi.org/10.3109/02770903.2013.803573).
35. Ogawa H, Fujimura M, Takeuchi Y, Makimura K. Role of fungal colonization for sensitization in asthma. *Clin Exp Allergy*. 2012;42(10):1540–1.
36. Ogawa H, Fujimura M, Takeuchi Y, Makimura K. The influence of *Schizophyllum commune* on asthma severity. *Lung*. 2011;189(6):485–92.
37. Ogawa H, Fujimura M, Takeuchi Y, Makimura K. Possible roles of 2 basidiomycetous fungi in allergic fungal respiratory disease. *J Allergy Clin Immunol*. 2012;130(1):279–80.