

K. Suzuki
H. Tanaka
H. Sahara
N. Tanaka
Y. Tamura
T. Naruse
H. Inoko
K. Tsushima
K. Kubo
S. Abe
N. Sato

HLA class II DPB1, DQA1, DQB1, and DRB1 genotypic associations with occupational allergic cough to Bunashimeji mushroom

Key words:
allergy; genotype; HLA; mushroom

Abstract: We previously reported that two-third of workers in a Bunashimeji mushroom (*Hypsizigus marmoreus*) farm complained of respiratory allergic symptoms, but one-third workers did not suffer from such symptoms even when working for a long period. CD4⁺ T-helper (Th) cells increased, and Th2/Th1 ratio increased in the allergic workers. To address these immunological backgrounds, we have investigated whether there is any relationship between mushroom allergy and human leukocyte antigen (HLA) class II alleles of DPB1, DQA1, DQB1, and DRB1 by using the polymerase chain reaction-restriction fragment length polymorphism (RFLP) and sequencing-based typing methods. We observed that the allele frequencies of DQA1*0103, DQB1*0601, and DRB1*0803 were significantly higher in the workers having no allergic symptoms than allergic workers (DQA1*0103: 57 vs 25%, DQB1*0601: 49 vs 14%, and DRB1*0803: 29 vs 0%). However, this phenomenon was not seen in workers producing another kind of mushroom, Honshimeji (*Lyophyllum aggregatum*). The HLA-DRB1*0803 allele alone, the DRB1*0803, DQA1*0103, DQB1*0601 haplotype, or both were negatively associated with allergy to Bunashimeji, and these alleles might be involved in the prevention of Bunashimeji mushroom-specific respiratory allergy.

Recently, respiratory and allergic diseases associated with exposure to some mushroom spores have been reported (1–6). Bunashimeji mushroom (*Hypsizigus marmoreus*) and Honshimeji (*Lyophyllum aggregatum*) are common edible mushrooms in Japan. These mushrooms are intensively cultivated in windowless rooms under high temperature and humidity in all seasons. When it is fully grown, the cultivating, harvesting, and packing rooms are filled with the mushroom spores, because the mushroom is 'open' at the very early stage, releasing the spores well before harvest. The diameter of the mushroom spore is 4–6 µm, and they can easily reach the alveoli of the lung. We previously reported hypersensitivity pneumonitis caused by Bunashimeji mushroom spore (7) and demonstrated two-third of workers complaining of severe allergic cough (>8 weeks) in the 2-year follow-up cross-sectional study (8). We also indicated that

Authors' affiliation:

K. Suzuki^{1,2,3},
H. Tanaka¹,
H. Sahara⁴,
N. Tanaka¹,
Y. Tamura²,
T. Naruse⁵,
H. Inoko³,
K. Tsushima⁵,
K. Kubo⁵,
S. Abe¹,
N. Sato²

¹Third Department of Internal Medicine, Sapporo Medical University School of Medicine, Hokkaido, Japan.

²First Department of Pathology, Sapporo Medical University School of Medicine, Hokkaido, Japan

³Department of Genetic Information, Division of Molecular Life Science, Tokai University School of Medicine, Kanagawa, Japan

⁴Marine Biomedical Institute, Sapporo Medical University School of Medicine, Hokkaido, Japan.

⁵First Department of Internal Medicine, Shinshu University School of Medicine, Nagano, Japan

Correspondence to:

Kazuhiko Suzuki, MD
Department of Genetic Information
Division of Molecular Life Science
Tokai University School of Medicine
Kanagawa 259-1193
Japan
Fax: +81 463 94 8884
e-mail: k-suzuki@hospital.hakodate.hokkaido.jp

Received date 10 November 2005, revised 4 February 2005, accepted 14 February 2005

Copyright © Blackwell Munksgaard 2005
doi: 10.1111/j.1399-0039.2005.00402.x

Tissue Antigens 2005; 65: 459–466
Printed in Singapore. All rights reserved

peripheral blood mononuclear cells, natural killer (NK) cells, and natural killer T (NKT) cells were significantly increased in the workers as compared with controls. Increases in CD4⁺ T cells, T-helper 2 (Th2) cells, Th2/1 ratio, and serum IL-13 levels and a decrease of serum IFN- γ were also found in the workers (9). On the other hand, one-third of the workers exposed to mushroom antigens had no allergic symptoms. From these backgrounds, individual susceptibility such as genetic factors to these allergic symptoms may exist other than environmental factors. In this respect, human leukocyte antigen (HLA) class II molecules are likely candidates for controlling the immune response to allergens. To address these immunological backgrounds, we have investigated whether there is any relationship between mushroom allergy and HLA class II genetic polymorphism in workers who are cultivating two different mushrooms.

Materials and methods

Subjects

A total of 164 workers were enrolled in this study. All subjects were working in cultivating or harvesting rooms in Bunashimeji or Honshimeji mushroom farm, and no workers had worked in another mushroom farm before. At first, a self-administered questionnaire asked for information of sex, age, smoking habits, previous allergic disorders, use of mask at work, duration of working at each mushroom farm, presence or absence of work-related cough, sputum, dyspnea, and time to onset of any symptoms.

Among 83 workers in a Bunashimeji mushroom farm, 12 with history of allergic disorders were excluded. Thus, we finally studied 71 workers (36 symptomatic, 35 asymptomatic). Symptomatic workers showed delayed hypersensitivity type of allergic symptoms, which occurred after work. All the subjects were unrelated Japanese (Wajin) living in Hokkaido located in the northernmost part of Japan.

Among 81 in a Honshimeji mushroom factory, 24 with other allergic disease were excluded. Thus 57 workers (35 symptomatic, 22 asymptomatic) were finally analyzed. All the subjects were also unrelated Japanese living in Nagano located in the center of Honshu island.

The subjects are defined as atopic when they have positive result for one or more of following 12 common antigens tested by the common kit (CAP Phadiatop FEIA, Pharmacia, Upjohn, Uppsala, Sweden); for mite (*Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*), cat hair, dog hair, cymodon, orchard grass, regweed, mugwort, white birch, Japanese cedar, *Candida*, and *Alternaria*.

For detecting serum IgE antibody binding to Bunashimeji mushroom antigens, Western blotting using anti-IgE antibody was performed in allergic and non-allergic workers. Results of

immunoblotting analysis of sera revealed IgE-binding proteins with molecular weights of about 30 kD in allergic workers in Bunashimeji mushroom farm. But these proteins were not identified in the sera from non-allergic subjects.

Methods

Blood samples were obtained from all the workers. Serum samples for immunologic studies were separated by centrifugation and stored at -80 °C until use. Genomic DNA was extracted from the peripheral blood leukocytes. Total serum IgE was measured in the 71 workers by the fluorescence enzymeimmunoassay method (UniCap Total IgE Diagnostic; Pharmacia).

This study was conducted with informed consent obtained from all the workers and approval by the ethics committee of Sapporo Medical University School of Medicine, Tokai University School of Medicine, and Shinshu University School of Medicine.

HLA typing

We performed DNA typing of HLA class II genes (HLA-DP, HLA-DQ and HLA-DR) in the workers with polymerase chain reaction using restriction fragment length polymorphism (PCR-RFLP) (10). In brief, extracted genomic DNA (100 ng) was amplified with each of 20 pm forward and reverse primer pairs by the PCR procedure with 2 units of Taq DNA polymerase. After amplification, aliquots (7 μ l) of the reaction mixture were digested with 2 units of each reaction endonuclease for 3 h after addition of appropriate incubation buffer. Digested products were electrophoresed on 12% polyacrylamide gels and detected by staining with ethidium bromide.

As a supplementary method for any significant HLA alleles, sequencing-based typing (SBT) was also employed by using the BigDyeTM Terminator Sequencing-Based Typing Kit, (Applied Biosystems, Foster city, CA). Briefly, at first, extracted genomic DNA (25 ng) was amplified with the specific PCR primer mixes (i.e., 11 PCR primer mixes; DR1, DR2, DR3/11/6, DR4, DR7, DR8/12, DR9, DR10, DRB3, DRB4, DRB5 for DRB typing) with 0.3 units of Taq DNA polymerase for low resolution sequence-specific primer (SSP)-PCR typing. Secondly, the positive amplification reactions from the SSP-PCR were used as sequencing templates to obtain high-resolution allele typing information. Finally the procedure for allele determination was performed using the MATCHTOOLS software and the MTNAVIGATOR software.

Statistical analysis

Phenotypic frequencies of HLA alleles were compared between the symptomatic workers' group and asymptomatic workers' group by

the χ^2 test and the Fisher exact test with 2×2 contingency tables. The corrected P values (P_c) were obtained by multiplying the P values by the number of comparisons made (i.e., $\times 50$ for DRB1 alleles at allelic level). The level of significance was set at 0.05.

Results

Population

Comparing all the workers investigated for HLA class II alleles, in the plant producing Bunashimeji mushroom in Hokkaido to all the workers in Honshimeji mushroom in Nagano, a significantly higher frequency of females in the group of the workers producing Bunashimeji mushroom (100 vs 81%, $P = 0.00012$) was observed. Also, a significant difference was found for age of the workers (41.9 ± 13.7 for the workers in Hokkaido vs 56.2 ± 9.8 for the workers in Nagano, $P = 0.00996$). Furthermore, significant differences between Hokkaido and Nagano were noted in the frequencies of smoking and mask wearing (smoking: 35% for the workers in Hokkaido vs 7% for the workers in Nagano, $P = 0.00010$; mask wearing: 85% for the workers in Hokkaido vs 33% for the workers in Nagano, $P < 0.000001$) (Table 1).

When each group of the workers was compared between allergic and non-allergic ones, no significant differences in sex, age, smoking, wearing mask and total serum IgE, except for atopy (48% for the allergic workers to Bunashimeji mushroom vs 25% for the non-allergic workers to Bunashimeji mushroom, $P = 0.0146$), and duration of working (66.8 ± 24.2 months for the allergic workers to Honshimeji mushroom vs 49.8 ± 20.7 months for the non-allergic workers to Honshimeji mushroom, $P = 0.01319$) were observed (Table 2).

Typing of HLA-DPB1, DQA1, DQB1, and DRB1

Bunashimeji mushroom

HLA class II alleles in the allergic and non-allergic workers were defined by the PCR-RFLP method.

Distribution of DPB1 alleles. No significant difference was found in the distribution of DPB1 alleles between the allergic workers and the non-allergic workers (data not shown).

Distribution of DQA1 alleles. As summarized in Table 3, the frequency of the DQA1*0103 allele was highly increased in the non-allergic workers when compared with the allergic workers ($P_c = 0.04558$).

Characteristics of workers, and comparison between Hokkaido and Nagano

	Workers in Hokkaido (<i>n</i> = 71)	Workers in Nagano (<i>n</i> = 57)	<i>P</i> ^c
Mushroom species cultivated	Bunashimeji (<i>Hypsizigus</i> <i>marmoreus</i>)	Honshimeji (<i>Lyophyllum</i> <i>aggregatum</i>)	
Sex (female/male) (female %)	71/0 (100)	46/11 (81)	0.00012
Age (years)	41.9 ± 13.7^a (20–61 ^b)	56.2 ± 9.8^a (31–70 ^b)	0.00996
Smoking (current or former) (%)	25/71 (35)	4/57 (7)	0.00010
Mask wearing during work (%)	60/71 (85)	19/57 (33)	<0.00001

^aMean $\pm$ SD.

^bRange.

^cFisher test for categorical variables and Student *t* test for continuous variables when required.

Table 1

Distribution of DQB1 alleles. The distribution of DQB1 alleles among the workers is summarized in Table 4. The frequency of the HLA-DQB1*0601 allele was significantly increased ($P_c = 0.02423$) in the non-allergic workers (49%) when compared with the allergic workers (14%).

Distribution of DRB1 alleles. As summarized in Table 5, the frequency of HLA-DRB1*0803 was significantly elevated ($P_c = 0.01988$) in the non-allergic workers (29%) when compared with the allergic workers (0%). This DRB1 allele determination by PCR-RFLP was confirmed by using the SBT method (BigDye™ Terminator Sequencing-Based Typing Kit, Applied Biosystems).

Honshimeji mushroom

In comparison with the DPB1, DQB1, and DRB1 allele frequencies determined by PCR-RFLP, no significant difference was found between the non-allergic and allergic workers. The frequency of DQA1*03 in the non-allergic workers was decreased ($P = 0.04983$); however, the difference did not reach the significant level after P -value correction (Tables 3–5).

Discussion

In this study, we found that the non-allergic workers in the Bunashimeji mushroom farm in Hokkaido were associated with HLA class II alleles, DQA1*0103, DQB1*0601 (DQ6), and DRB1*0803 (DR8).

Characteristics of workers, and comparison between Hokkaido and Nagano

	Workers in Hokkaido (Bunashimeji)		<i>P</i> ^c	Workers in Nagano (Honshimeji)		<i>P</i> ^c
	Allergic (<i>n</i> = 36)	Non-allergic (<i>n</i> = 35)		Allergic (<i>n</i> = 35)	Non-allergic (<i>n</i> = 22)	
Sex (female/male)	36/0	35/0	NS	27/8	19/3	NS
Age (years)	44.2 ± 11.8 ^a (20–61 ^b)	41.5 ± 19.1 ^a (18–61 ^b)	NS	58.1 ± 8.3 ^a (39–72 ^b)	53.0 ± 11.2 ^a (31–70 ^b)	NS
Duration of working (months)	58.1 ± 38.8 ^a (13–106 ^b)	43.5 ± 29.5 ^a (13–106 ^b)	NS	66.8 ± 24.2 ^a (18–168 ^b)	49.9 ± 20.7 ^a 6–72 ^b	0.01319
Smoking (current or former) (%)	15/36 (42)	10/35 (80)	NS	2/35 (6)	2/22 (8)	NS
Mask wearing during work (%)	32/36 (89)	28/35 (80)	NS	12/35 (34)	7/22 (32)	NS
Total serum IgE (IU/ml)	157.4 ± 199.9	131.1 ± 343.3	NS	ND	ND	
Atopy (%)	14/25 (48)	6/24 (25)	0.0146	ND	ND	
Onset of symptoms (months)	2.8 ± 2.1 ^a (1–6 ^b)	–		14.4 ± 24.6 ^a (1–120 ^b)	–	
Cough (%)	32/36 (89)	0/35 (0)		25/35 (71)	0/22 (0)	
Sputum (%)	7/36 (19)	0/35 (0)		25/35 (71)	0/22 (0)	
Dyspnea (%)	6/36 (17)	0/35 (0)		2/35 (6)	0/22 (0)	

NS, not significant; ND, not done.

^aMean ± SD.^bRange.^cFisher test for categorical variables and Student *t* test for continuous variables when required.**Table 2**

From these results, two possible explanations are considered; 1) HLA phenotype may influence directly preventing of the Bunashimeji mushroom allergy through possible different interactions between the HLA class II molecules and the spore allergens; 2) other gene(s) (non-HLA gene) controlling the T-cell response of mushroom allergy could be in linkage disequilibrium with HLA class II markers and be maintained in the population depending in the history of recombination events. We did not analyze the second hypothesis in this study. However, it has been recently reported that the HLA class II region is making the second hypothesis very unlikely (11). It was referred that CD4/8 ratios have genetic regulation in humans (12). When considering the first hypothesis, it is well known that in Japanese subjects (Wajin) a strong linkage disequilibrium exists, mainly between DRB1*0803, DQA1*0103, and DQB1*0601 (13). But in Japanese, DQB1*0601 is similarly known to be in a strong linkage disequilibrium with DRB1*1502 (DR2/15) (13). In this study, the allele frequencies of DQB1*0601 and DRB1*0803 were significantly higher in the non-allergic workers than in the allergic workers, but not true for DRB1*1502 in the Hokkaido farm. If DQB1*0601 is a directly associative allele, both the allele frequencies of DRB1*0803 and *1502 should be elevated in the non-allergic workers due to linkage disequilibria. It is suggested that the DRB1*0803 (DR8) allele alone, the DRB1*0803, DQA1*0103, DQB1*0601 haplotype, or both are strongly associated with the inhibition of Bunashimeji mushroom allergy. Negative associations between HLA-DR alleles and allergic diseases have been reported for associations between DR15 and

gelatin allergy (14), between DR4 and mountain cedar pollinosis (15), and between DR4 and bee venom allergy (16). But the immunological mechanism underlying the negative associations between DR and allergic diseases has not been defined in detail. It is reported that the immune response to Japanese cedar is regulated by the HLA-DQ locus controlling suppressor activity, and that this suppression can be eliminated *in vitro*, through depletion of CD8-bearing lymphocytes (17–19). Furthermore, the recent study by Matsuoka et al. (20) demonstrated that DR-restricted T cells which were reactive with mite antigens exhibited a higher IFN- γ /IL-4 ratio than DQ- or DP-restricted T cells. It has been also speculated that the DR-peptide complex delivers the strongest avidity between T cell receptor (TCR), leading to Th1-prone immune responses (21, 22). Similarly, in Bunashimeji mushroom allergy, HLA-DRB1*0803 may define a suppressor T-cell population or lead to Th1-prone responses. Thus, HLA-DRB1*0803 molecules may exert protective effect against the allergic response. The molecular mechanism of HLA-DR involvement in protecting subjects from Bunashimeji mushroom allergy awaits further investigation.

Honshimeji mushroom is also known to cause respiratory allergic diseases like hypersensitivity pneumonitis (23). The discrepancy in HLA class II allele associations in the non-allergic workers between Bunashimeji and Honshimeji mushroom may be relevant to the different influence of sex, age, smoking, the ethnic origin of populations, the sensitization state of the workers, and/or mushroom species between Hokkaido and Nagano. Because the workers in mushroom

Distribution of HLA-DQA1 alleles between allergic and non-allergic workers

DQA1 allele	Hokkaido (Bunashimeiji)						Nagano (Honshimeiji)					
	Allergic (n = 36)			Non-allergic (n = 35)			Allergic (n = 35)			Non-allergic (n = 22)		
	n	%		n	%	χ^2	n	%		n	%	χ^2
DQA1 allele												
DQA1*0101/*0104 ^a	14	39		10	29	0.84	5	14		4	18	0.15
DQA1*0102	12	33		10	29	0.19	12	34		9	41	0.25
DQA1*0103	9	25		20	57	7.59	14	40		4	18	2.42
DQA1*03	29	81		21	60	3.60	22	63		19	86	3.70
DQA1*0401	0	0		2	6	2.12	1	3		0	0	0.64
DQA1*05	7	19		3	9	1.73	9	26		2	9	2.40
DQA1*0601	0	0		1	3	1.04	0	0		0	0	—

NS, not significant; Pc, corrected P values.
^aDQA1*0101 and *0104 could not be differentiated.
Frequencies were compared between two groups by the χ^2 test and the Fisher exact test with 2 × 2 contingency tables. The corrected P values (Pc) were obtained by multiplying the P values by the number of comparisons made.

Table 3

Distribution of HLA-DQB1 alleles between allergic and non-allergic workers

DQB1 allele	Hokkaido (Bunashimeiji)						Nagano (Honshimeiji)						
	Allergic (n = 36)			Non-allergic (n = 35)			Allergic (n = 35)			Non-allergic (n = 22)			
	n	%		n	%	Statistical tests	n	%	Pc	n	%	Statistical tests	
						χ^2			P			χ^2	P
													Pc
DQB1*0301	10	28		6	17	1.15	NS	9	26	2	9	2.40	NS
DQB1*0302	11	31		6	17	1.75	NS	8	23	5	23	0.00	NS
DQB1*0303	11	31		6	17	1.75	NS	10	29	9	41	0.93	NS
DQB1*0401	8	22		11	31	0.77	NS	5	14	6	27	1.47	NS
DQB1*0402	3	8		2	6	0.19	NS	2	6	3	14	1.06	NS
DQB1*0501	4	11		1	3	1.85	NS	4	11	1	5	0.80	NS
DQB1*0502	1	3		3	9	1.12	NS	0	0	3	14	5.04	NS
DQB1*0503	7	19		4	11	0.87	NS	6	17	1	5	1.99	NS
DQB1*0601	5	14		17	49	9.98	0.00162	14	40	4	18	2.98	NS
DQB1*0602/*0603 ^a	5	14		9	26	1.57	NS	4	11	4	18	0.51	NS
DQB1*0604	3	8		4	11	0.19	NS	4	11	5	23	1.30	NS

NS, not significant; Pc, corrected P values.
^aDQB1*0602 and *0603 could not be differentiated.
Frequencies were compared between two groups by the χ^2 test and the Fisher exact test with 2 × 2 contingency tables. The corrected P values were obtained by multiplying the P values by the number of comparisons made.

Table 4

Distribution of HLA-DRB1 alleles between allergic and non-allergic workers

DRB1 allele	Hokkaido (Bunashimeji)							Nagano (Honshimeji)						
	Allergic (n = 36)		Non-allergic (n = 35)		Statistical tests			Allergic (n = 35)		Non-allergic (n = 22)		Statistical tests		
	n	%	n	%	χ^2	P	Pc	n	%	n	%	χ^2	P	Pc
DRB1*0101	4	11	1	3	1.85	NS	NS	2	6	0	0	1.30	NS	NS
DRB1*0401	0	0	0	0	–	–	–	2	6	0	0	1.30	NS	NS
DRB1*0403	3	8	2	6	0.19	NS	NS	2	6	5	23	3.63	NS	NS
DRB1*0405	7	19	10	29	0.81	NS	NS	6	17	8	36	2.69	NS	NS
DRB1*0406	4	11	0	0	4.12	NS	NS	2	6	1	5	0.34	NS	NS
DRB1*0407	1	3	1	3	0.00	NS	NS	0	0	0	0	–	–	–
DRB1*0410	4	11	1	3	1.85	NS	NS	2	6	1	5	0.04	NS	NS
DRB1*0802	0	0	2	6	2.12	NS	NS	2	6	0	0	1.30	NS	NS
DRB1*0803	0	0	10	29	11.97	0.00040	0.01988	5	14	0	0	3.45	NS	NS
DRB1*0901	15	42	11	31	0.80	NS	NS	12	34	9	41	0.25	NS	NS
DRB1*1101	2	6	4	11	0.79	NS	NS	4	11	0	0	2.70	NS	NS
DRB1*1201	5	14	3	9	0.50	NS	NS	0	0	1	5	1.62	NS	NS
DRB1*1202	0	0	1	3	1.04	NS	NS	0	0	0	0	–	–	–
DRB1*1302	3	8	2	6	0.19	NS	NS	4	11	5	23	1.30	NS	NS
DRB1*1401	3	8	3	9	0.00	NS	NS	3	9	4	18	1.16	NS	NS
DRB1*1403	2	6	0	0	2.00	NS	NS	1	3	1	5	0.11	NS	NS
DRB1*1405	4	11	1	3	1.85	NS	NS	4	11	1	5	2.70	NS	NS
DRB1*1406	2	6	1	3	0.32	NS	NS	3	9	0	0	1.99	NS	NS
DRB1*1407	0	0	1	3	1.04	NS	NS	0	0	0	0	–	–	–
DRB1*1501	7	19	9	26	0.40	NS	NS	6	17	4	18	0.01	NS	NS
DRB1*1502	3	8	7	20	2.00	NS	NS	8	23	4	18	1.60	NS	NS
DRB1*1602	2	6	0	0	2.00	NS	NS	0	0	0	0	–	–	–

NS, not significant:

Frequencies were compared between two groups by the χ^2 test and the Fisher exact test with 2×2 contingency tables. The corrected P values (Pc) were obtained by multiplying the P values by multiplying the number of comparisons made.

Table 5

farm producing Bunashimeji in Hokkaido were all females, the hormonal environment may be an additional relevant factor modulating the T-cell response. The difference of age between two groups was also found in this study. Recently, age-dependent genes regulating T-cell subpopulations have been described in mice (24). Furthermore, the frequency of the smoking workers between two plants was significantly different. It is well known that non-smoking is significantly associated with allergic alveolitis in hypersensitivity pneumonitis (25). However, the number of the subjects studied may be not large enough to explain them. Interestingly, we observed that these allergic symptoms disappeared when workers moved to other mushroom farms, such as Eringi mushroom (*Pleurotus eringi*) and Maitake mushroom (*Grifola frondosa*). The results obtained from the present study showed that the onset of allergic

symptoms to Bunashimeji mushroom spore appeared significantly earlier when compared with Honshimeji mushroom. Tsushima et al. (23) reported that Honshimeji mushroom spore caused 10 cases of hypersensitivity pneumonitis, but only 2 patients were induced to hypersensitivity pneumonitis by Bunashimeji mushroom spore. These results may suggest that the antigenicity of Bunashimeji mushroom spore is quite different from that of Honshimeji mushroom spore. Therefore, mushroom allergy seemed to be strictly dependent on the mushroom species.

In summary, the present data strongly indicate that in the workers with no allergy to Bunashimeji mushroom, the DRB1*0803 alone, the DRB1*0803, DQA1*0103, DQB1*0601 haplotype, or both are a strong genetic factor controlling no allergic immune response to its spore.

References

1. Sanderson W, Kullman G, Sastre J et al. Outbreak of hypersensitivity pneumonitis among mushroom farm workers. *Am J Ind Med* 1992; **22**: 859–72.
2. Moinard J, Vergeret J, Tunon de Lara JM et al. A new form of mushroom workers' lung. *Rev Mal Respir* 1991; **8**: 308–10.
3. Kamm YJL, Folgering HT, van den Bogart HGG, Cox A. Provocation test in extrinsic allergic alveolitis in mushroom workers. *Neth J Med* 1991; **38**: 59–64.
4. Matsui S, Nakazawa T, Umegae Y, Mori M. Hypersensitivity pneumonitis induced by shiitake mushroom spores. *Intern Med* 1992; **31**: 1204–6.
5. Ishii M, Kikuchi A, Kudoh K et al. Hypersensitivity pneumonitis induced by inhalation of mushroom (*Pholiota nameko*) spores. *Intern Med* 1994; **33**: 683–5.
6. Inage M, Takahashi H, Nakamura H, Masakane I, Tomoike H. Hypersensitivity pneumonitis induced by spores of *Pholiota Nameko*. *Intern Med* 1996; **35**: 301–4.
7. Tanaka H, Sugawara H, Saikai T, Tsunematsu K, Takahashi H, Abe S. Mushroom worker's lung caused by spores of *Hypsizygus marmoreus* (Bunashimeji). *Chest* 2000; **118**: 1506–9.
8. Tanaka H, Saikai T, Sugawara H et al. Workplace-related chronic cough on a mushroom farm. *Chest* 2002; **122**: 1080–5.
9. Saikai T, Tanaka H, Sato N, Abe S, Matsuura A. Mushroom plant workers experience a shift towards a T helper type 2 dominant state: contribution of innate immunity to spore antigen. *Clin Exp Immunol* 2004; **135**: 119–24.
10. Inoko H, Ota M. PCR-RFLP. In: Bidwell J, Hui, KM, eds. Handbook of HLA Typing Techniques. Boca Ration, FL: CRC Press, 1993: 9–70.
11. The MHC Sequencing Consortium (Aguado B, Bahram S, Beck S et al). Complete structure and gene map of human major histocompatibility complex (MHC). *Nature* 1999; **401**: 921–3.
12. Amadori A, Zamarchi R, De Silvestro G et al. Genetic control of the CD4/8 T-cell ratio in humans. *Nat Med* 1995; **1**: 1279–83.
13. Saito S, Ota S, Yamada E, Inoko H, Ota M. Allele frequencies and haplotypic associations defined by allelic DNA typing at HLA class I and class II loci in the Japanese population. *Tissue Antigens* 2000; **56**: 522–9.
14. Sakaguchi M, Nakayama T, Kaku H et al. Analysis of HLA in children with gelatin allergy. *Tissue Antigens* 2002; **59**: 412–6.
15. Reid MJ, Nishi WA, Whisman BA et al. HLA-DR4-associated nonresponsiveness to mountain-cedar allergen. *J Allergy Clin Immunol* 1992; **89**: 593–8.
16. Lympny P, Kemeny DM, Weish KI, Lee TH. An HLA-associated nonresponsiveness to mellitin: a component of bee venom. *J Allergy Clin Immunol* 1990; **86**: 160–70.
17. Sasazuki T, Nishimura Y, Muto M, Saito Y. HLA-linked genes controlling immune response and disease susceptibility. *Immunol Rev* 1983; **70**: 51–75.
18. Matsushita S, Muto M, Suemura M, Saito Y, Sasazuki T. HLA-linked nonresponseveness to cryptomerla japonica pollen allergy. *J Immunol* 1987; **138**: 109–15.
19. Hirayama K, Matsushita S, Kikuchi I, Inoue M, Ohta N, Sasazuki T. HLA-DQ is epistatic to HLA-DR in controlling the immune response to schistosomal antigen in humans. *Nature* 1987; **327**: 426–30.
20. Matsuoka T, Tabata H, Matsuoka S. Monocytes are differentially activated through HLA-DR-DQ, and -DP molecules via mitogen activated protein kinases. *J Immunol* 2001; **166**: 2202–8.
21. Foey AD, Parry SL, Williams LM, Feldmann M, Foxwell BM, Brennan FM. Regulation of monocyte IL-10 synthesis by endogenous IL-1 and TNF- α : role of the p38 and p42/44 mitogen-activated protein kinases. *J Immunol* 1998; **160**: 920–8.
22. Hsieh CS, Macatonia SE, Tripp CS, Wolf SF, O'Garra A, Murphy KM. Development of Th1 CD4⁺ T cells through IL-12 produced by *Listeria*-induced macrophages. *Science* 1993; **260**: 547–9.
23. Tsushima K, Fujimoto K, Yamazaki Y et al. Hypersensitivity pneumonitis induced by spores of *Lyophyllum aggregatum*. *Chest* 2001; **120**: 1085–93.
24. Jackson AU, Galecki AT, Burke DT, Miller RA. Genetic polymorphisms in mouse genes regulating age-sensitive and age-stable T cell subsets. *Genes Immun* 2003; **4**: 30–9.
25. Warren CP. Extrinsic allergic alveolitis; a disease commoner in non-smokers. *Thorax* 1977; **32**: 567–9.