

HLA DPB1*0201 allele is negatively associated with immunoglobulin E responsiveness specific for house dust mite allergens in Taiwan

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Summary

Background House dust mite (HDM) *Dermatophagoides pteronyssinus* is the most important source of indoor allergens that cause allergic diseases in Taiwan. We prepared purified HDM allergens (Der p 1, Der p 2 and Der p 5) to detect allergen-specific immunoglobulin (Ig) E responsiveness among a large number of test subjects. The robust genetic typing system for HLA class II genes also facilitated the study on association of HLA and allergic response toward HDM.

Objective This study intended to investigate the association between HLA class II alleles and the IgE responsiveness to the major allergens from HDM, *D. pteronyssinus*.

Methods Two hundred and forty-eight subjects were selected for HLA association study. Plasma HDM allergen (Der p 1, Der p 2, Der p 5) -specific IgE and Der p 2-specific IgG antibodies were detected by ELISA, while HLA class II -DRB1, -DQA1, -DQB1, -DPB1 genetic polymorphism was determined by polymerase chain reaction/sequence-specific oligonucleotide probe hybridization (PCR/SSOPH). Statistical comparison of the allelic distribution of each HLA class II genes among the individuals with/without HDM allergen-specific IgE and IgG antibodies were performed.

Results There was no significant association between HLA DRB1, DQB1, DQA1 alleles and HDM-specific IgE responsiveness noted. Only DRB1*0803 and the linked DQA1*0103 alleles showed positive association with Der p 5-specific IgE responsiveness. However, we found that HLA-DPB1*1301 predisposed subjects to IgE responsiveness to HDM Der p 5. HLA DPB1*0501 was weakly associated with the IgE responsiveness to HDM Der p 1 and Der p 5. There was a strong negative association between the HLA-DPB1*0201 allele with IgE responsiveness to Der p 1 (OR: 0.30, $P \leq 0.0001$, $P_c \leq 0.0007$, $P_c \leq 0.010$).

Conclusion We clearly observed the association between HLA DPB1 alleles and specific IgE responsiveness to HDM major allergens. The molecular mechanism of HLA-DPB1*0201 involvement in protecting subjects from HDM-specific IgE responsiveness awaits further investigation.

Keywords: allergen-specific immunoglobulin E, Der p 1, Der p 2, Der p 5, HLA class II genes, house dust mite (HDM), PCR/SSOPH

Clinical and Experimental Allergy, Vol. 30, pp. 538–545. Submitted 7 April 1999; revised 10 June 1999; accepted 12 July 1999.

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Introduction

In Taiwan, house dust mite (HDM) is the most important source of indoor allergen that causes atopic diseases such as allergic asthma, allergic rhinitis and atopic dermatitis [1].

The presence of allergen-specific immunoglobulin (Ig) E antibodies is implicated in the sensitization of mast cells and the pathogenesis of unique inflammatory response-associated allergic manifestations. A positive skin test to allergen correlates well with the presence of specific IgE, which was commonly used for clinical diagnosis in allergic diseases. Prevalence of allergic disorders, such as asthma and allergic rhinitis among school-age children in Taipei was reported to be 26.5% [2]. Most asthmatic sera showed specific IgE antibodies to mite antigen Der p 1 and Der p 2 in Western blot analysis [3]. Skin test performed by using recombinant Der p 1, Der p 2 and Der p 5 and immunoblotting for HDM-specific IgE also revealed that Der p 2 is the most important allergen causing allergic asthma in Northern Taiwan [3,4]. Der p 2-specific IgE could be detected in up to 80% or more of the sera from asthma and allergic rhinitis patients. House dust mite antigens are ubiquitous, however, not all individuals exposed to HDM antigens developed allergy. There is familial aggregation of allergic asthma [5]. Twin studies also showed that the concordance rate for asthma is higher in monozygotic than in dizygotic twins [6]. Taken together, the previous reports suggested certain genetic control might predispose an individual to allergy. A likely candidate for controlling specific IgE response is the major histocompatibility complex (MHC), the human leucocyte antigens (HLAs) [7]. The cell immune response depends on the interaction of processed antigenic peptide presented on HLA class II antigen and T cell receptor, with the development of TH2-skewed responses. TH2 cells secrete interleukin (IL) -4 and IL-13 to help B cells to produce specific IgE. HLA class II molecules may also play important role in determining TH1/TH2 development in the allergic response [8].

Previous attempts to find an association between allergic asthma and HLA antigens did not obtain conclusive results due to several inherent problems or weaknesses associated with the design of the various studies [9–14]. In the current study, availability of purified major HDM allergens, Der p 1, Der p 2 and Der p 5, allowed convenient detection for allergen-specific IgE responsiveness in a large sample of randomly selected subjects. The advent of a robust genetic typing system for HLA class II alleles further facilitated the study on the association of HLA and allergic immune response. The subjects selected for this study are Taiwanese, descendants of Chinese immigrants from the south-eastern provinces of the mainland China some hundred years ago. The major populations ($\approx 85\%$) are of Fukinese and Hakka ethnic groups, approximately 13% are recent immigrants from other parts of mainland China. It has been shown by previous study that the allelic distribution of HLA class II genes is very similar among this homogenous population [15].

Materials and methods

Subjects

Four hundred and eighty-six unrelated young adults (20–22 years-old) were recruited in a students' physical examination program at National Taiwan University. A questionnaire concerning if presence of (1) manifestation of clinical symptoms and signs of allergies such as asthma or allergic rhinitis, and (2) family history of allergy, was filled. A simple physical examination was carried-out and the blood sample was collected. None of the selected students was a foreigner or of aboriginal ethnic background. No subject claimed receiving immunotherapy due to allergy. As most of the HDM-sensitive subjects in Taiwan have detectable Der p 2-specific serum IgE antibodies [3,4], the plasma samples were firstly subjected to detection of Der p 2-specific IgE. One hundred and fifteen of the 486 subjects screened had detectable Der p 2-specific IgE; One hundred and thirteen Der p 2-positive and another 135 Der p 2-negative subjects were selected for the HLA association study. Two Der p 2-positive subjects were excluded because their DNA samples were not available. Out of the 135 Der p 2-negative subjects selected in the association study, 49 were selected on the basis of self-claimed allergic status, the remainder 86 were randomly selected. All the 248 selected subjects were subsequently screened for serum IgE specific for Der p 1 and Der p 5. Due to logistic constraint, no further study was performed with the 220 unselected subjects.

Sample preparation

EDTA-anticoagulated blood was collected from each selected subject. The plasma was kept at -20°C for detection of HDM allergen-specific antibodies. Genomic DNA was prepared from the leucocyte portion by the salting-out method [16], and stored at -20°C for genetic typing of HLA class II genes.

Detection of HDM allergen-specific IgE and IgG antibodies by ELISA method

The plasma samples of the selected subjects were further analysed for Der p 1-, Der p 2- and Der p 5-specific IgE and Der p 2-specific IgG antibodies. Recombinant Der p 2 and Der p 5 were prepared from *Escherichia coli* [4,17]. Native Der p 1 was purified from spent mite medium with monoclonal antibody (4C1) affinity chromatography [18]. The HDM allergens were coated on 96-well ELISA plates ($0.4\text{ }\mu\text{g/well}$ in $100\text{ }\mu\text{L}$ of 0.1 mol/L NaHCO_3 , pH 8.2) and blocked in 3% BSA overnight. For detecting Der p 2 and Der p 5-specific IgE, $100\text{ }\mu\text{L}$ of diluted plasma (1 : 2 dilution in PBS/1% BSA) was incubated with the allergen for 12 h.

Table 1. Correlation of allergen-specific IgE antibodies and allergic status among the 248 selected students

Status*	Anti-Der p 1(%)	Anti-Der p 2(%)	Anti-Der p 5(%)	Total
All	107 (43)	113 (46)	89 (36)	248
NA	34 (27)	39 (31)	25 (20)	125
AR/AR**	48 (52)	48 (52)	40 (43)	93
AS/AS**	8 (80)	8 (80)	8 (80)	10
AR + AS (AS**)	17 (85)	18 (90)	16 (80)	20

Status*: the self-awareness of allergic symptoms according to the questionnaires completed by the students. All: NA + AR + AS, NA: no allergic symptoms, AR: allergic rhinitis, with family history, AS: asthma, with family history, AR**, AS**: suspected symptoms and signs, but without family history.

The specific IgE antibodies were detected by 1:2000 diluted biotin-labelled mouse antihuman IgE (PharMingen, San Diego, CA, USA) followed by 1:2000 diluted extra-vidin alkaline phosphatase (ALP) conjugate and pNPP substrate (Sigma, St Louis, MO, USA). Extensive washing with PBS buffer containing 0.05% Tween-20 was performed following each incubation step. The A405 nm for each sample was compared with those of the cord blood sera that serve as negative controls. The sample with A405 nm five times greater than the average absorbance of negative controls was scored as positive. Plasmas were diluted 1:5 for the detection of Der p 1-specific IgE. For detecting Der p 2-specific IgG, 1:100 diluted plasmas were applied, secondary antibody goat antihuman IgG-ALP conjugate (Sigma) and PNPP substrate were used for signal development.

Genetic typing of HLA class II DRB1, DQA1, DQB1 and DPB1 genes by polymerase chain reaction/sequence-specific oligonucleotide probe hybridization (PCR/SSOPH)

The genetic typing by PCR/SSOPH was performed by methods previously described [19–22]. Briefly, the second exons of HLA class II genes encoding the most polymorphic sequences were amplified by specific primer sets. The PCR products were denatured and immobilized onto nylon membranes. Panels of 5'-biotinylated SSO probes [19–22] were used to detect the hypervariable regions within the polymorphic exons. The genotype for each class II locus was constructed for each DNA sample.

Statistics

All the subjects were grouped according to presence of detectable IgE specific for HDM allergens (Der p 1, Der p 2 or Der p 5) or not, and their allergic status (see below). The gene frequencies of each class II allele of the individuals within each group were compared by Chi-square test. The odds ratio (OR) of each allele predisposing IgE

responsiveness to HDM allergens was calculated accordingly by Wolf's or Haldane's formula. A P_c value (p corrected for the number of alleles observed in each locus examined) less than 0.05 was considered to be statistically significant.

Results

Detection of Der p 1-, Der p 2- and Der p 5-specific IgE antibodies among the selected subjects

A group of 248 subjects (123 males and 125 females) were selected for this study. Subjects were regarded as allergic if they were aware of allergic symptoms as asthma or allergic rhinitis and had a positive family history among their first and second-degree relatives. The correlation of allergic status and the presence of Der p 1-, Der p 2-, and Der p 5-specific IgE antibodies among the 248 selected individuals were shown in Table 1. Fifty percent (125/248) of the selected students were not aware of any allergic symptom and signs (NA), 20–30% of them had detectable IgE specific for Der p 1, Der p 2, or Der p 5. For students suffered allergic rhinitis only, 52% had Der p 1 or Der p 2-specific IgE and 43% had Der p 5-specific IgE. Eighty to 90 percent of the students with asthmatic history had detectable HDM allergen-specific IgE. Detection of Der p 2-specific IgG showed that both the Der p 2-specific IgE positive and negative individuals had Der p 2-specific IgG with comparably high antibody titres (data not shown).

Association of HLA class II alleles with allergen-specific IgE responsiveness

By using 30 SSOPs for DRB1, 12 for DQA1, 17 for DQB1 and 25 for DPB1 gene, more than 95% of the class II alleles observed in Taiwan could be resolved [23]. The genotypes of these class II loci were determined for each of the 248 subjects. Allelic frequencies of each class II gene were

Table 2 Allelic distribution of the HLA class II DRB1 gene among individuals with/without Der p 1-, Der p 2- and Der p 5-specific IgE in Taiwan

Class II allele	IgE to:					
	Der p 1		Der p 2		Der p 5	
	(+) N = 214 n (%)	(-) N = 282 n (%)	(+) N = 226 n (%)	(-) N = 270 n (%)	(+) N = 178 n (%)	(-) N = 318 n (%)
DRB1*						
0101	1 (0.0)	0 (0.0)	0 (0.0)	1 (0.4)	0 (0.0)	1 (0.3)
0301	15 (7.0)	23 (8.2)	16 (7.1)	22 (8.2)	10 (5.6)	28 (8.8)
0401	1 (0.5)	2 (0.7)	1 (0.4)	2 (0.7)	1 (0.6)	2 (0.6)
0403	6 (2.8)	10 (3.6)	7 (3.1)	9 (3.3)	5 (2.8)	11 (3.5)
0405	11 (5.1)	18 (6.4)	12 (5.3)	17 (6.3)	9 (5.1)	20 (6.3)
0406	8 (3.7)	8 (2.8)	8 (3.5)	8 (3.5)	7 (3.9)	9 (2.8)
0407	0 (0.0)	1 (0.4)	0 (0.0)	1 (0.4)	0 (0.0)	1 (0.3)
0408	0 (0.0)	1 (0.4)	0 (0.0)	1 (0.4)	1 (0.6)	0 (0.0)
0701	7 (3.3)	6 (2.1)	7 (3.1)	6 (2.2)	4 (2.3)	9 (2.8)
0801	0 (0.0)	1 (0.4)	0 (0.0)	1 (0.4)	0 (0.0)	1 (0.3)
0802	3 (1.4)	3 (1.1)	4 (1.8)	2 (0.7)	3 (1.7)	3 (0.9)
0803	19 (8.9)	28 (9.7)	20 (8.8)	27 (10.0)	23 (12.9)	24 (7.6)*
0901	31 (14.5)	37 (13.1)	34 (15.1)	34 (12.6)	28 (15.7)	40 (12.6)
1001	3 (1.4)	2 (0.7)	2 (0.9)	3 (1.1)	2 (1.1)	3 (0.9)
1101	19 (8.9)	23 (8.2)	18 (8.0)	24 (8.9)	13 (7.3)	29 (9.1)
1104	1 (0.5)	0 (0.0)	0 (0.0)	1 (0.4)	0 (0.0)	1 (0.3)
1201	3 (1.4)	12 (4.3)	4 (1.8)	11 (4.1)	3 (1.7)	12 (3.8)
1202	19 (8.9)	29 (10.3)	20 (8.8)	28 (10.4)	14 (7.9)	34 (10.7)
1301	1 (0.5)	1 (0.4)	1 (0.4)	1 (0.4)	2 (1.1)	0 (0.0)
1302	6 (2.8)	7 (2.5)	4 (1.8)	9 (3.3)	4 (2.2)	9 (2.8)
1305	7 (3.3)	1 (0.4)	8 (3.5)	0 (0.0)	6 (3.4)	2 (0.6)
1312	0 (0.0)	2 (0.7)	0 (0.0)	2 (0.7)	0 (0.0)	2 (0.6)
1401	13 (6.1)	11 (3.9)	13 (5.8)	11 (4.1)	9 (5.1)	15 (4.7)
1405	3 (1.4)	4 (1.4)	5 (2.2)	2 (0.7)	3 (1.7)	4 (1.3)
DR2	37 (17.)	52 (18.4)	42 (18.6)	47 (17.4)	31 (17.4)	58 (18.2)

*Odds ratio: 1.82, 95% confidence interval: 1.0–3.3, $P \leq 0.05$, P_c : non-significant

calculated and the association between the class II loci and allergen-specific IgE responsiveness was examined (Tables 2, 3 and 4). Analysis of results indicated there was no obvious association between DRB1 (Table 2), DQA1 and DQB1 (Table 3) alleles and HDM-specific IgE responsiveness. There was an insignificant increase in the frequency of DRB1*0803 allele within IgE responders to Der p 5. The frequency of DQB1*0502 was slightly higher among individuals with HDM allergen-specific IgE antibodies. The DQA1*0103 was the only allele found to be positively associated with Der p 5-specific IgE responsiveness (OR: 2.28, $P \leq 0.005$, $P_c \leq 0.04$).

Interestingly, DPB1*1301, *0501 and DPB1*0201 alleles were associated with allergen-specific IgE

(Table 4). It was noted that DPB1*1301 predisposed subjects to Der p 1-, Der p 2- and Der p 5-specific IgE responsiveness, although only in the case of Der p 5 IgE, the association reached statistical significance (OR: 2.73, $P \leq 0.0016$, $P_c \leq 0.018$). The DPB1*0501 allele showed a positive association with Der p 1- and Der p 5-specific IgE responsiveness. This association turned out to be insignificant after the P -values were corrected. Intriguingly, we found that the DPB1*0201 allele was negatively associated with specific IgE production to Der p 1 (OR: 0.30, $P \leq 0.0001$, $P_c \leq 0.001$), Der p 2 (OR: 0.46, $P \leq 0.004$, $P_c \leq 0.056$) and Der p 5 (OR: 0.35, $P \leq 0.0007$, $P_c \leq 0.010$). Furthermore, as all the individuals tested were positive for Der p 2-specific IgG irrespective of the

Table 3. Allelic distribution of HLA class II DQA1, DQB1 genes among individuals with/without Der p 1-, Der p 2- and Der p 5-specific IgE in Taiwan

Class II allele	IgE to:					
	Der p 1		Der p 2		Der p 5	
	(+) <i>N</i> = 214 <i>n</i> (%)	(-) <i>N</i> = 282 <i>n</i> (%)	(+) <i>N</i> = 226 <i>n</i> (%)	(-) <i>N</i> = 270 <i>n</i> (%)	(+) <i>N</i> = 178 <i>n</i> (%)	(-) <i>N</i> = 318 <i>n</i> (%)
DQB1*						
0201	22 (10.3)	28 (9.9)	23 (10.8)	27 (10.0)	14 (7.9)	36 (11.3)
0301	48 (22.4)	62 (22.0)	49 (21.7)	61 (22.6)	35 (19.7)	75 (23.6)
0302	17 (7.9)	22 (7.8)	18 (8.0)	21 (7.8)	14 (7.9)	25 (7.9)
0303	31 (14.5)	39 (13.8)	34 (15.0)	36 (13.3)	28 (15.7)	42 (13.2)
0401	9 (4.2)	17 (6.0)	10 (4.4)	16 (5.9)	9 (5.1)	17 (5.4)
0402	3 (1.4)	4 (1.4)	4 (1.8)	3 (1.1)	3 (1.7)	4 (1.3)
0501	8 (3.7)	5 (1.8)	4 (1.8)	9 (3.3)	5 (2.8)	8 (2.5)
0502	28 (13.1)	27 (9.6)	30 (13.3)	25 (9.3)	23 (12.9)	32 (10.0)
05031	10 (4.7)	13 (4.6)	14 (6.3)	9 (3.4)	9 (5.1)	14 (4.4)
0504	0 (0.0)	1 (0.4)	0 (0.0)	1 (0.4)	0 (0.0)	1 (0.3)
0601	24 (11.2)	43 (15.2)	25 (11.1)	42 (15.6)	27 (15.2)	40 (12.6)
0602	7 (3.3)	14 (5.0)	10 (4.4)	11 (4.1)	5 (2.8)	16 (5.0)
0603	1 (0.5)	1 (0.4)	1 (0.4)	1 (0.4)	2 (1.1)	0 (0.0)
0609	6 (2.8)	6 (2.1)	4 (1.8)	8 (3.0)	4 (2.2)	8 (2.5)
DQA1*						
0101	26 (12.2)	22 (7.8)	25 (11.1)	23 (8.5)	16 (9.0)	32 (10.0)
0102	38 (17.8)	58 (20.6)	25 (11.1)	23 (8.5)	32 (18.0)	64 (20.1)
0103	21 (9.8)	29 (10.3)	22 (9.7)	28 (10.4)	27 (15.2)	23 (7.2)*
0201	6 (2.8)	7 (2.5)	7 (3.1)	6 (2.2)	4 (2.2)	9 (2.8)
0301	57 (26.6)	75 (26.6)	62 (27.4)	70 (25.9)	51 (28.6)	81 (25.5)
0401	2 (0.9)	4 (1.4)	3 (1.3)	1 (1.1)	2 (1.1)	4 (1.3)
0501	45 (21.0)	66 (23.4)	47 (20.8)	64 (23.7)	32 (18.0)	79 (24.8)
0601	19 (8.9)	21 (7.4)	18 (8.0)	22 (8.2)	14 (7.9)	26 (8.2)

*Odds ratio: 2.28, 95% confidence interval: 1.27–4.09, $P \leq 0.005$, $P_c \leq 0.039$

presence of Der p 2-specific IgE, the DPB1 alleles were associated with HDM allergen-specific IgE but not IgG responsiveness.

Discussion

Numerous studies have been performed to address the question about the association between allergic asthma and the possible predisposing genetic factor [24], especially the polymorphic HLA molecules. However, most of the previous studies failed to prove a clear and significant association of allergic asthma and the HLA class I and class II molecules. There are several confounding factors and inherent problems in experimental designs that made the interpretation of association between HLA and allergic asthma and other disorders difficult. For example, the

demographic pictures and the allelic distribution of HLA varied in the study groups of different ethnic background. In addition, in most of the previous studies, the allergens that caused asthma were poorly defined. Moreover, the traditional microcytotoxicity test [25] used for determining the HLA class II polymorphism had inherent problems, such as requirement of viable B cells, expensive typing antisera that showed batch-to-batch variation and a large number of alleles remain undefined.

HDM's importance as an allergen source in Taiwan is well documented [1]. Elevated levels of allergen-specific serum IgE is an important marker of atopic diseases. In the present study, a total of 486 unrelated young adults (aged 20–22 years) were recruited as study subjects. The atopic status of the study subjects was defined according to their awareness of allergic symptoms and a positive family

Table 4. Allelic distribution of HLA class II DPB1 genes among individuals with/without Der p 1-, Der p 2- and Der p 5-specific IgE in Taiwan

Class II allele	IgE to:					
	Der p 1		Der p 2		Der p 5	
	(+) <i>N</i> = 214 <i>n</i> (%)	(-) <i>N</i> = 282 <i>n</i> (%)	(+) <i>N</i> = 226 <i>n</i> (%)	(-) <i>N</i> = 270 <i>n</i> (%)	(+) <i>N</i> = 178 <i>n</i> (%)	(-) <i>N</i> = 318 <i>n</i> (%)
DPB1*						
0101	0 (0.0)	1 (0.4)	0 (0.0)	1 (0.4)	1 (0.6)	0 (0.0)
0201	14 (6.5)	54 (19.2)*	20 (8.8)	48 (17.9)†	12 (6.7)	56 (17.6)‡
0202	13 (6.1)	12 (4.3)	13 (5.8)	12 (4.4)	8 (4.5)	17 (5.4)
0301	13 (6.1)	16 (5.7)	16 (7.1)	13 (4.8)	11 (6.2)	18 (5.7)
0401	14 (6.5)	22 (7.8)	16 (7.1)	20 (7.4)	13 (7.3)	23 (7.2)
0402	5 (2.3)	9 (3.2)	4 (1.8)	10 (3.7)	4 (2.2)	10 (3.1)
0501	115 (53.7)	117 (41.5)§	114 (50.4)	118 (43.7)	94 (52.8)	138 (43.4)¶
0901	2 (0.9)	3 (1.1)	2 (0.9)	3 (1.1)	3 (1.7)	2 (0.6)
1301	23 (10.8)	18 (6.4)**	24 (10.6)	17 (6.3)††	24 (13.5)	17 (5.4)‡‡
1401	3 (1.4)	12 (4.3)	2 (0.9)	13 (4.8)	1 (0.6)	14 (4.4)
1701	3 (1.4)	3 (1.1)	2 (0.9)	4 (1.5)	1 (0.6)	5 (1.6)
1901	2 (0.9)	8 (2.8)	3 (1.3)	7 (2.6)	1 (0.6)	9 (2.8)
2101	6 (2.8)	4 (1.4)	6 (2.6)	4 (1.5)	4 (2.3)	6 (1.9)
4801	1 (0.5)	1 (0.4)	2 (0.9)	0 (0.0)	1 (0.6)	1 (0.4)
Not done		2	2			2

*Odds ratio: 0.30, 95% confidence interval: 0.16–0.56, $P \leq 0.0001$, $P_c \leq 0.001$. †Odds ratio: 0.46, 95% confidence interval: 0.26–0.79, $P \leq 0.004$, $P_c \leq 0.056$ (NS). ‡Odds ratio: 0.35, 95% confidence interval: 0.18–0.66, $P \leq 0.0007$, $P_c \leq 0.010$. §Odds ratio: 1.64, 95% confidence interval: 1.14–2.34, $P \leq 0.007$, $P_c \leq 0.095$ (NS). ¶Odds ratio: 1.49, 95% confidence interval: 1.03–2.15, $P \leq 0.044$, P_c : (NS). **Odds ratio: 1.75, 95% confidence interval: 0.93–3.32, $P \leq 0.08$ (NS). ††Odds ratio: 1.75, 95% confidence interval: 0.92–3.33, $P \leq 0.08$ (NS). ‡‡Odds ratio: 2.73, 95% confidence interval: 1.44–5.20, $P \leq 0.0016$, $P_c \leq 0.022$.

history. Purified recombinant HDM allergens, Der p 1, Der p 2 and Der p 5, were used to test the allergen-specific IgE responsiveness. The HLA class II genotypes of these subjects were studied by the robust PCR/SSOPH system. The results showed that for those subjects suffering atopic disorders, the house dust mite was the most important source of allergens (Table 1). As the prevalence of Der p 1- and Der p 2-specific IgE antibodies was comparable with previously reported incidence of asthma and allergic rhinitis in Taiwan [1], indicating that the subjects we recruited indeed represent a typical sample of Taiwanese population.

We found that the DPB1*1301 and *0501 alleles predisposed subjects to HDM allergens Der p 1-, Der p 2- and Der p 5-specific IgE responsiveness. Most interestingly, the DPB1*0201 allele was significantly associated with IgE non-responsiveness to HDM allergens in Taiwanese. As all subjects could produce similar level of Der p 2-specific IgG irrespective of their corresponding IgE production, we concluded that the HLA class II alleles cited above were associated with HDM allergen-specific IgE but not IgG

responsiveness. HLA class II alleles such as DRB1*0803 and DQA1*0103 were differentially distributed among HDM allergen-specific IgE-positive and -negative subjects, although the differences were statistically insignificant in most of the cases.

Previous reports revealed a positive association between asthma and DR4, DR7 in France [12]. The Taiwanese have different DR4 alleles from those of the Caucasoid populations. The major Taiwanese DR4 alleles are DRB1*0403, *0405 and *0406 in contrast with *0401 in Caucasians. The DRB1*0401 allele differs from other DR4 alleles in its lysine residue at the 57th position of DR β chain. The DRB1*0401 also linked to different DQA1-DQB1 haplotypes from the other DR4 alleles. The DR7 allele is uncommon (gene frequency ~2%) in Taiwanese and we did not find similar association between these class II alleles and HDM-specific IgE responsiveness. Further, a previous study showed that HLA-DQw2 was excessive, while DR4 tended to be lower among asthmatic children in Taiwan [9]. We did not find similar association between DR4 types, the

DR4 suballeles, or DQB1*0201 and the HDM allergen-specific IgE responsiveness. It is important to note that the previous Taiwanese study used a microcytotoxicity test to determine HLA-DR and -DQw specificity, leaving 32 and 68% of DR and DQ alleles unresolved. The unresolved alleles might stand for a homozygosity or be due to lack of appropriate typing sera for special HLA types, and thus made the statistics less confident.

A negative association between asthma and DPB1*0401 in the South American Mollato population has been reported [11]. The DPB1*0401 is the most dominant DPB1 allele in the Caucasoid populations, while being moderately common among Taiwanese. We did not find difference in DPB1*0401 gene frequency between our study groups. Interestingly, the protective DPB1*0201 noted in Taiwanese shares amino acid sequences at the polymorphic region at residues 7–10 (LFQG) and 85–88 (GGPM) in the outer extracellular domain of DP β chain. In fact, the DPB1*0201 differs from the DPB1*0401 allele by only four residues within the DP β extracellular outer domain of about 100 amino acids.

Studies in the UK failed to demonstrate association between asthma or Der p 1- and Der p 2-specific IgE responsiveness and HLA class II alleles [13,14]. In these studies, families containing asthmatic children were recruited. Comparing class II alleles among families by their IgE seropositivity might mask a possible association, since class II alleles were inherited among the study subjects, while the population control used contained 10–20% of allergic individuals which could also confound the statistical analysis.

It is of particular interest that a single DPB1*0201 allele was negatively associated with Der p 1, Der p 2, and Der p 5 allergen-specific IgE responsiveness. These three allergens are molecules of different size, structure and biological activity [26]. Aron *et al.* also noticed HLA DR4 and 7 might be associated with asthmatic patients sensitive to multiple allergens [12]. It is intriguing that HLA-DP alleles are found to be significantly associated with IgE responsiveness, as the HLA-DP is the least expressed class II antigen on antigen-presenting cells. Although there were reports indicating that HLA-DP could serve as restriction element for T-cell activation [27]. The molecular mechanisms underlying HLA-DP involvement in predisposing to or protecting subjects from HDM allergen-specific IgE responsiveness await further investigation. Another possibility is there may exist (an)other gene(s) closely linked to the DPB1 locus, which can direct the diversification of TH cell immune response against mite allergens.

Recently, Moffatt *et al.* [28] reported a strong allelic association between T-cell receptor VA8.1*2 allele and reactivity to Der p 2. High IgE responders to Der p 2 was confined to subjects who were positive for both

TCRVA8.1*2 and HLA-DRB1*1501. This study strongly suggested germline TCR α gene and HLA class II antigen interact to restrict the response to exogenous antigens. It is clear that in studying genetic factors that determined the nature of immune response toward allergens in different ethnic populations, the polymorphism of HLA molecules/germline TCR genes, and the molecular characteristics of the allergens should all be taken into consideration.

Acknowledgements

This work was fully supported by the Research Grant NTUH M86–205 from National Taiwan University Hospital. We would like to thank our colleagues in the Department of Paediatrics, NTUH, for their help in performing the medical examination and questionnaire design. We would like to thank Dr Luis Carabolla for his helpful comments and discussion.

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