

Positive association between HLA-DRB1*07 and specific IgE responses to purified major allergens of *D. pteronyssinus* (Der p 1 and Der p 2)

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Background and Objective: Human leukocyte antigen (HLA) class II gene products are involved in the antigen presentation of exogenous antigens. The aim of this study was to evaluate whether specific immunoglobulin (Ig)E responses to purified major allergens of *Dermatophagoides pteronyssinus* (Der p 1 and Der p 2) were associated with genotypes of HLA-DRB1 alleles.

Subjects and Methods: Peripheral venous blood samples were collected from two groups of unrelated Korean adolescents: 168 subjects with positive skin response (wheal ≥ 3 mm) to crude *D. pteronyssinus* allergens and 94 age-matched controls with negative skin response and low serum-specific IgE to crude *D. pteronyssinus* allergens. The former group was found to contain 104 with high serum-specific IgE to crude *D. pteronyssinus* allergens, 100 with high serum-specific IgE to Der p 1, and 122 with high serum-specific IgE to Der p 2. Genotypes of the HLA-DRB1 alleles were determined using polymerase chain reaction-sequence specific oligonucleotide probes.

Results: HLA-DRB1*07 was significantly higher in subjects with a high serum-specific IgE response to crude *D. pteronyssinus* allergens than the controls (16.3% vs 3.2%, odds ratio [OR] = 5.93, corrected P [Pc] = 0.02). The excess of DRB1*07 was more marked in those with high serum-specific IgE responses to Der p 1 or Der p 2 than the controls (20.0% vs 3.2%, OR = 7.58, P_c = 0.004; 18.9% vs 3.2%, OR = 7.05, P_c = 0.006). Among subjects with high serum IgE to Der p 1, DRB1*13 significantly increased than the controls (32.0% vs 13.8%, OR = 2.93, P_c = 0.03).

Conclusions: We clearly observed the association between HLA-DRB1 alleles and specific IgE responsiveness to *D. pteronyssinus* major allergens. The molecular mechanism of HLA-DRB1*07 and DRB1*13 involvement in *D. pteronyssinus*-specific IgE responsiveness awaits further investigation.

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INTRODUCTION

House-dust mites (HDM), particularly *Dermatophagoides pteronyssinus* (DP)

and *D. farinae*, are important sources of allergens inside homes in humid areas worldwide.^{1,2} Sensitization to HDM has shown to be of particular importance in the development of asthma and bronchial hyperresponsiveness in children and adolescents.^{3,4} There are clear indications that expo-

sure to allergens increases the risk of specific sensitizations to allergens such as HDM, providing evidence on the importance of environmental factors.⁵ There are, however, two outstanding molecular complexes in which genetic variants might significantly contribute to a differential risk of sensitization to allergens. These are the major histocompatibility complex (MHC), in particular the class II MHC molecules, and the T-cell receptor complex. Both are crucially involved in the cognate interactions between antigen-presenting cells and the T-helper cells.

Human leukocyte antigen (HLA) loci have been extensively investigated in relation to immunoglobulin (Ig)E responses.⁶ Following the original observations of Levine et al,⁷ a number of genetic association studies have been conducted which have investigated the relationships between particular HLA class I and II types in case-control studies of specific allergen responsiveness.^{8,9} When low-molecular-weight, highly purified and characterized sub-fractions of allergens were investigated, association were shown with class II types; elevated IgE levels to short ragweed (*Ambrosia artemisiifolia*) pollen allergens Amb a 5, Amb t 5, and Amb p 5 have been documented with HLA-DR2/Dw2. However, significant associations have not been found between genotypes of the HLA genes and the major allergens of HDM. Therefore, our study was designed to investigate whether there are associations between genotypes of HLA-DRB1 alleles and

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IgE responses to highly purified major allergens (Der p 1 and Der p 2) of HDM.

METHODS

Subjects

One hundred sixty-eight adolescents with positive skin responses to DP (wheal formed by DP >3 mm) and 94 age-matched nonatopic healthy controls were recruited for this study. They were unrelated subjects living in rural areas of Cheju Island, Korea. The mean age of the DP-sensitive subjects was 14.1 years (range 13 to 16), 90 were female and 78 were male. One hundred four (61.9%) of the positive skin responders had high serum-specific IgE levels to crude DP allergens. One hundred (59.5%) and 122 (72.6%) of the positive skin responders had high specific IgE levels to Der p 1 and Der p 2, respectively. The mean age of the controls was 14.2 (range 13 to 16) years, 44 were female and 40 were male. They had been exposed to the same environment as the DP-sensitive subjects since infancy, had no asthmatic or rhinitis symptoms, and showed negative skin test responses to any aeroallergen tested (mean wheal size by any allergen <2 mm). Individuals were genotyped for HLA-DRB1 alleles using polymerase chain reaction with sequence specific oligonucleotide probes (PCR-SSOP). All subjects gave informed consent, and the study protocol was approved by the Ethics Committee of the Seoul National University Hospital.

Skin Prick Tests

All the subjects underwent skin prick tests using a panel of 11 common aeroallergens (Allergopharma, Hamburg, Germany). None of the subjects had received antihistamines orally for 5 days before the study. The panels obtained commercially were: *D. pteronyssinus*, *D. farinae*, cat fur, molds (*Alternaria* spp. and *Aspergillus* spp.), pollens (two mixtures of early blooming trees and late blooming trees, grass pollen mixture, mugwort, and ragweed), and cockroach. A positive control of histamine (1 mg/mL, Allergopharma), along with a negative control of diluent (Allergopharma), was in-

cluded in all tests. Skin prick tests were performed on the volar side of both forearms. Fifteen minutes after the skin prick, the mean diameter of a wheal formed by the allergen was measured. If the wheal size formed by DP was equal to or larger than 3 mm, the reaction was defined as positive.

ELISA for Specific IgE

The serum-specific IgE levels induced by crude DP allergens, Der p 1 and Der p 2 were determined using ELISA. Microtiter plates (Dynatech, Grafton, OH) were coated with 50 μ L/well (5 μ g/well) of crude DP extract, Der p 1, and Der p 2 (all from Allergopharma). The absorbance (optical density) was read at 410 nm by an automated microplate reader. All assays were performed in duplicate. The cutoff values of high specific IgE bindings to crude DP extract, Der p 1, and Der p 2 were determined from the mean plus twice the standard deviation of the absorbance of the control subjects.

Genotypes of HLA-DRB1 Alleles:

PCR-SSOP

Genomic DNA from the peripheral blood mononuclear cells of the positive skin responders and the controls was extracted using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI).

HLA-DRB1 alleles were genotyped using the Dynal RELI SSOP HLA-DRB test (Dynal AS, Oslo, Norway). PCR amplification of the second exon of the DRB1 gene was performed as directed by the supplier. Three milliliters of prewarmed hybridization buffer (50° C) was added to each well, and 70 μ L denatured amplified sample or control was added. Hybridization was performed at 30 g for 30 minutes using specific oligonucleotide probes, as directed by the supplier. HLA-DRB1 antigens were interpreted using the Dynal FELI SSOP Pattern Matching Computer Program.

Statistical Analysis

HLA-DRB1 alleles were determined by direct counting. Genotype (allele) frequency (%) was calculated as follows:

Genotype frequency = $n/N \times 100$

n = number of individuals positive

for each genotype

N = total number of individuals tested

The allele frequencies were compared using the χ^2 test. When either value was <5, Fisher's exact test was used. P -values were corrected for the number of comparisons (P_c) which were considered significant when $P_c < 0.05$.

RESULTS

Genotypes of HLA-DRB1 alleles in subjects with positive skin responses to crude DP allergens are illustrated in Table 1. We found an excess of the HLA-DRB1*07 in the 168 positive skin responders compared with a control group of 94 unselected and age-matched individuals [16.1% compared with 3.2% in controls, OR (odds ratio) = 5.81 (95% CI 2.95 to 11.43), $P_c = 0.02$]. The relative risk of DRB1*07 in the positive skin responders compared with the controls was 5.04. However, there were no differences between the two groups in terms of the distribution of DRB1*01, *02, *03, *04, *08, *09, *10, *11, *12, *13, or *14.

Table 2 shows the allelic frequency of HLA-DRB1 gene in subjects with high serum-specific IgE response to crude DP allergens. The allelic frequency of DRB1*07 was significantly higher in those with high serum-specific IgE than in the controls (16.3% versus 3.2%, OR=5.93 [95% confidence interval, or CI, 2.75 to 12.77], $P_c = 0.02$). The relative risk of DRB1*07 in the positive skin responders compared with the controls was 5.12, which is similar to the excess of DRB1*07 in the subjects sensitive to skin prick testing to DP. However, no significant difference was found in the distributions of the other HLA-DRB1 genotypes in the two groups.

Allelic frequencies of the HLA-DRB1 gene in subjects with high serum IgE responses to Der p 1 and Der p 2 are illustrated in Tables 3 and 4, respectively. We found an excess of

the DRB1*07 in those with high Der p 1-specific IgE levels than in the controls (20.0% vs 3.2%, OR = 7.58 [95% CI 3.68 to 15.6], $P_c = 0.004$). The allelic frequency of DRB1*07 was also higher in those with high Der p 2-specific IgE levels than in the controls (18.9% vs 3.2%, OR = 7.05 [95% CI 3.42 to 14.51], $P_c = 0.006$). The relative risk of DRB1*07 in those with high serum IgE responses to Der p 1 and Der p 2 compared with the controls is 6.27 and 5.91, respectively. The excess of DRB1*07 in the DP-sensitive subjects was more prominent in those who had high serum-specific IgE to Der p 1 and Der p 2. An increase of DRB1*13 and a small decrease of DRB1*14 was found among those with high Der p 1-specific IgE (32% compared with 13.8% in controls, OR = 2.93 [95% CI 1.63 to 5.26], $P_c = 0.03$; 13% compared with 23.4% in controls, $P = 0.05$, $P_c > 0.05$). There were no differences in the distribution of the allelic frequencies of the other HLA-DRB1 genotypes.

DISCUSSION

Our results reveal that subjects with high serum-specific IgE as well as positive skin-test responses to crude DP extract had an excess of DRB1*07, which was more remarkable in those with high serum-specific IgE to highly purified allergens of DP such as Der p 1 and Der p 2. Among subjects with high serum-specific IgE to Der p 1, there was also an increase in DRB1*13 compared with the controls.

HLA genes play a major role in the regulation of immune response. In addition, the polymorphisms of these loci play an important role in their predisposition to a large number of immunologically mediated diseases. The HLA class II (HLA-DR, DQ, and DP) molecules are heterodimers (α and β chains) with a high proportion of polymorphisms.¹⁰ With the exception of the HLA-DRA molecule, the genes encoding the functional class II molecules are highly polymorphic, with virtually all the variability localized on the second exon. The HLA-DRB1 gene is the most polymorphic of the human MHC

Table 1. Allelic Frequency of HLA-DRB1 Gene in Subjects with Positive Skin Response to Crude *D. pteronyssinus* Allergens

Alleles	Skin responders* (n = 168)		Nonatopic controls (n = 94)		OR (95% CI)	P	Pc†
	No.	GF (%)	No.	GF (%)			
DRB1*01	28	16.7	19	20.2	5.81 (2.95–11.43)	NS	NS
DRB1*02	33	19.6	22	23.4		NS	NS
DRB1*03	10	6.0	3	3.2		NS	NS
DRB1*04	49	29.2	33	35.1		NS	NS
DRB1*07	27	16.1	3	3.2		0.002	0.02
DRB1*08	44	26.2	21	22.3		NS	NS
DRB1*09	35	20.8	19	20.2		NS	NS
DRB1*10	3	1.8	1	1.1		NS	NS
DRB1*11	8	4.8	4	4.3		NS	NS
DRB1*12	23	13.7	13	13.8		NS	NS
DRB1*13	38	22.6	13	13.8		NS	NS
DRB1*14	24	14.3	22	23.4		NS	NS

GF: genotype frequency; NS: not significant; OR: odds ratio.

* Subjects with positive skin response to crude *D. pteronyssinus* allergens (wheal ≥ 3 mm).

† P corrected for the number of alleles (12) tested.

Table 2. Allelic Frequency of HLA-DRB1 Gene in Subjects with High Serum-Specific IgE Response to Crude *D. pteronyssinus* Allergens

Alleles	IgE responders* (n = 104)		Nonatopic controls (n = 94)		OR (95% CI)	P	Pc†
	No.	GF (%)	No.	GF (%)			
DRB1*01	16	15.4	19	20.2	5.93 (2.75–12.77)	NS	NS
DRB1*02	23	22.1	22	23.4		NS	NS
DRB1*03	5	4.8	3	3.2		NS	NS
DRB1*04	31	29.8	33	35.1		NS	NS
DRB1*07	17	16.3	3	3.2		0.002	0.02
DRB1*08	21	20.2	21	22.3		NS	NS
DRB1*09	20	19.2	19	20.2		NS	NS
DRB1*10	4	3.8	1	1.1		NS	NS
DRB1*11	4	3.8	4	4.3		NS	NS
DRB1*12	14	13.5	13	13.8		NS	NS
DRB1*13	23	22.1	13	13.8		NS	NS
DRB1*14	18	17.3	22	23.4		NS	NS

GF: genotype frequency; NS: not significant; OR: odds ratio.

* Subjects with high serum-specific IgE response to crude *D. pteronyssinus* allergens.

† P corrected for the number of alleles (12) tested.

class II genes, and more than 120 distinct alleles have been identified, making it a powerful marker for individual identification.¹⁰ Therefore, we targeted the HLA-DRB1 gene in this study.

In an effort to elucidate the role of immune response genes in the development of atopic disease, many population studies have examined the association between HLA-class II alleles and atopic responses to a number of

different allergens. These studies often yielded conflicting results because of selection method of cases and controls, sample size, and differences in ethnic background. However, the Koreans are relatively homogeneous in the ethnic background. In particular, the DP-sensitive adolescents and the age-matched control subjects recruited in this study were from the unrelated general population living in rural areas on Cheju Island,

Table 3. Allelic Frequency of HLA-DRB1 Gene in Subjects with High Serum-Specific IgE Response to *Der p 1* Allergen

Alleles	<i>Der p 1</i> responders* (n = 100)		Nonatopic controls (n = 94)		OR (95% CI)	P	Pc†
	No.	GF (%)	No.	GF (%)			
DRB1*01	12	12.0	19	20.2		NS	NS
DRB1*02	19	19.0	22	23.4		NS	NS
DRB1*03	4	4.0	3	3.2		NS	NS
DRB1*04	33	33.0	33	35.1		NS	NS
DRB1*07	20	20.0	3	3.2	7.58 (3.68–15.6)	0.0003	0.004
DRB1*08	21	21.0	21	22.3		NS	NS
DRB1*09	21	21.0	19	20.2		NS	NS
DRB1*10	2	2.0	1	1.1		NS	NS
DRB1*11	4	4.0	4	4.3		NS	NS
DRB1*12	13	13.0	13	13.8		NS	NS
DRB1*13	32	32.0	13	13.8	2.93 (1.63–5.26)	0.003	0.03
DRB1*14	13	13.0	22	23.4		NS	NS

GF: genotype frequency; NS: not significant; OR: odds ratio.

* Subjects with high serum-specific IgE response to *Der p 1* allergen.

† P corrected for the number of alleles (12) tested.

Table 4. Allelic Frequency of HLA-DRB1 Gene in Subjects with High Serum-Specific IgE Response to *Der p 2* Allergen

Alleles	<i>Der p 2</i> responders* (n = 122)		Nonatopic controls (n = 94)		OR (95% CI)	P	Pc†
	No.	GF (%)	No.	GF (%)			
DRB1*01	18	14.8	19	20.2		NS	NS
DRB1*02	24	19.7	22	23.4		NS	NS
DRB1*03	5	4.1	3	3.2		NS	NS
DRB1*04	35	28.7	33	35.1		NS	NS
DRB1*07	23	18.9	3	3.2	7.05 (3.42–14.51)	0.0005	0.006
DRB1*08	28	23.0	21	22.3		NS	NS
DRB1*09	26	21.3	19	20.2		NS	NS
DRB1*10	2	1.6	1	1.1		NS	NS
DRB1*11	6	4.9	4	4.3		NS	NS
DRB1*12	15	12.3	13	13.8		NS	NS
DRB1*13	27	22.1	13	13.8		NS	NS
DRB1*14	21	17.2	22	23.4		NS	NS

GF: genotype frequency; NS: not significant; OR: odds ratio.

* Subjects with high serum specific IgE response to *Der p 2* allergen.

† P corrected for the number of alleles (12) tested.

where they have lived in relatively the same environment since infancy.

Following the original observations of Levine et al,⁷ a number of genetic association studies have been conducted to investigate the relationships between particular HLA class II types and specific allergen responsiveness in case-control studies.^{8,9,11,12} When low-molecular-weight, highly purified and characterized subfractions of allergen

were investigated, associations were shown between HLA class II types and elevated IgE levels to short ragweed (*Ambrosia artemisiifolia*) pollen allergens. Other purified allergens, which have been found to be associated with HLA class II allergens include rye grass (Lop p 1) and olive (Ole e 1).^{9,13} However, previous population association studies demonstrated a conflicting results between particular HLA

class II alleles and IgE reactivity to the HDM allergen.^{14–18} For example, Hu et al¹⁸ reported no significant association between HLA DRB1 alleles and HDM-specific IgE responsiveness in Taiwan populations.

The negative effects of HLA polymorphism on the risk of sensitization to whole complex allergens such as crude DP allergens may be attributable to the many epitopes associated with complex allergens. DP is a source of numerous allergens, and extracts have been shown to contain as many as 32 different IgE-binding components, as evaluated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting.¹⁹ The most prominent allergens, particularly Der P 1 and Der p 2, from DP have been the subject of extensive biochemical, molecular, and immunologic characterization.^{20,21} If HLA-DRB1 alleles define IgE response to DP, they presumably must do so by restricting the response to a single critical oligopeptide derived from the specific allergen. In this study, the positive association with DRB1*07 was observed in subjects with high specific IgE response to highly purified allergens (Der p 1 and Der p 2), as well as in those with high specific IgE to crude DP allergens. Further, among those with high specific IgE to Der p 1, there was also an excess of HLA-DRB1*13. These findings suggest that the HLA-DRB1*07 allele may restrict immune response to oligopeptide derived from Der p 1 and Der p 2, and DRB1*13 from Der p 1. However, this association study has a limitation that the apparent linkage to the HLA-DRB1 alleles may in fact have represented a linkage disequilibrium to other nearby genes on chromosome 6p, such as HLA-DQ and tumor necrosis factor genes.

CONCLUSION

We clearly observed the association between HLA-DRB1 alleles and specific IgE responsiveness to *D. pteronysinus* major allergens. However, the molecular mechanism of HLA-DRB1*07 and DRB1*13 involvement in *D. ptero-*

nyssinus-specific IgE responsiveness awaits further investigation.

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