

Association between HLA class II locus and the susceptibility to *Artemisia* pollen-induced allergic rhinitis in Chinese population

MIN WANG, MD,* ZHI-MIN XING, MS,* DE-LIN YU, MD, PHD, ZHENG YAN, MS, and LI-SHENG YU, MD, PHD, Beijing, People's Republic of China

OBJECTIVE: The aim of the study was to determine whether susceptibility or resistance to *Artemisia* pollen-induced allergic rhinitis was associated with HLA class II *DQA1*, *DQB1* loci.

STUDY DESIGN AND SETTING: Forty-one subjects with allergic rhinitis and 41 healthy controls from Beijing were genotyped at HLA class II *DQA1*, *DQB1* alleles by polymerase chain reaction amplification with sequence-specific primers-based technique.

RESULTS: The allele frequencies of *HLA-DQA1*0201*, *DQB1*0602* were lower in patients with allergic rhinitis compared with the controls (24.39% versus 46.34%, $P = 0.038$; 4.88% versus 26.83%, $P = 0.007$), and the frequency of *DQA1*0302* was higher among patients than the controls (58.54% versus 14.63%, $P = 0.00004$, $P_c = 0.0004$).

CONCLUSION AND SIGNIFICANCE: *HLA-DQA1* and *-DQB1* genes may be involved in the development of *Artemisia* pollen-induced allergic rhinitis. *HLA-DQA1*0201*, *DQB1*0602* alleles may be a protective factor and *DQA1*0302* may be a susceptible factor for *Artemisia* pollen-induced allergic rhinitis. (Otolaryngol Head Neck Surg 2004;130:192-6.)

Allergic rhinitis is a multifactorial disease, the pathogenesis of which relates to genetics, immunology, the environment, and other factors. It has been found that this disease has very obvious genetic predisposition.¹ The key role of IgE and T cells in allergic diseases suggests that immune

responses genes are important candidates. As the one of the main immune molecule, HLA class II (*HLA-DR*, *-DQ*, and *-DP*) genes are located on chromosome 6p and are expressed as highly polymorphic glycoproteins on some antigen presenting cell (dendritic cells, Langerhans cells, et al) surfaces. Allergens are mostly soluble, low-molecular-weight proteins that generally enter the body through the mucosa via inhalation. The HLA class II molecule can bind to the exogenous peptides (such as inhaled allergens) to form an MHC-peptide complex. In the form of peptide-MHC class II complexes, some of the allergen is probably internalized by antigen presenting cells, which then migrate to the local nodes and present antigen to CD4⁺ T cells. Allergen-specific B-lymphocytes are activated by interaction with allergens and by T cell help to proliferate and differentiate and to switch to production and secretion of IgE antibodies.

Having a critical role in the immune recognition of foreign antigens, most of the loci within HLA class II are highly polymorphic; that is, they possess many different genetic variants (alleles). It seems probable that this polymorphism is associated with important survival advantages for the human species, including an increased diversity of immune function. Because of the pivotal role of the HLA class II molecules, and in particular in regulating the immune response and its polymorphism, it have been implicated in susceptibility to a wide range of diseases known to have an immunologic basis. Allergic disease is no exception. HLA has been reported to be associated with several allergens in allergic asthma such as Amb aV of ragweed and dust mite allergens.² In this study, we wanted to investigate the distribution of *DQA1*, *DQB1* genes in *Artemisia* pollen-sensitive allergic rhinitis patients in Beijing to find the association of the genotypes *DQA1*, *DQB1*

From the Department of ENT, People's Hospital, Peking University.

*These authors contributed equally to the work.

This study was supported by the 985 Scientific Foundation of Peking University Medical Center for Human Disease Genomics (grant 2001-5).

Reprint requests: Li-Sheng Yu, MD, PhD, Department of ENT, People's Hospital, Peking University, 11 South Xizhimen St, Beijing, PR China 100044; e-mail, lishengyu6@hotmail.com.

0194-5998/\$30.00

Copyright © 2004 by the American Academy of Otolaryngology-Head and Neck Surgery Foundation, Inc.

10.1067/j.otohns.2003.08.012

with allergic rhinitis, through which we want to study the pathogenesis of allergic rhinitis at genetic level.

SUBJECTS AND METHODS

Subjects

Forty-one *Artemisia* pollen-sensitive allergic rhinitis patients with obvious autumn onset and 41 nonatopic healthy controls were recruited for this study. They were unrelated subjects living in urban areas of Beijing. The mean age of the patients was 38.3 years (age range, 20 to 63 years; 17 men and 24 women); 19 had asthma, and all were from the outpatient department of our hospital. All of the patients completed the questionnaires and tests, which included allergic rhinitis history, drug and food sensitivity history, kindred hypersensitivity history, asthmatic history, intracutaneous tests, nasal mucosal scraping mast cell counting or nasal discharge eosinophil counting, and sIgE antibody specific to *Artemisia* pollen measured by CAP system. The diagnosis of allergic rhinitis was based on the criteria of the Workshop Report of ARIA (Allergic Rhinitis and Its Impact on Asthma).³ Briefly, the diagnosis of allergic rhinitis was made based on chronic nasal obstruction or congestion, itchy nose, rhinorrhea, sneezing, lack of chronic infection, nasal examination, and allergy tests (intracutaneous tests, serum-specific IgE, nasal mucosal scraping mast cell counting, or nasal discharge eosinophil counting). Forty-one non-symptomatic healthy control subjects were physical examination people in our hospital. They had been exposed to the same environment as the *Artemisia* pollen-sensitive subjects. The mean age of the patients was 42.45 years (Age range, 26 to 60 years; 22 men and 19 women). A positive history of allergy in the subjects or their family was excluded by questionnaire. All of the subjects are of Han nationality. Hypertension, rheumatism, diabetes, and other diseases related to HLA had been excluded. All of the subjects gave informed consent, and the study protocol was approved by the Ethics Committee of the Peking University Center for Human Disease Genomics.

Methods

Intracutaneous tests. All of the subjects underwent intracutaneous testing using a panel of 15

common inhalant allergens (Peking Union Medical College Hospital, China). The allergens included *Artemisia* pollen, autumn and summer pollen (not including *Artemisia* pollen), house dust, *Dermatophagoides pteronyssinus* (Der p), and other. A positive control of histamine dihydrochloride, used at a concentration of 1 mg/L, along with a negative control of diluents was included in every skin test study. Intracutaneous tests were performed on the volar side. None of the subjects had received antihistamines or steroid for 1 week preceding the study. Test was considered positive if the maximum wheal diameter was greater than 10 mm.

Specific serum IgE. Serum specific IgE antibodies against *Artemisia* pollen were measured by fluorescent enzyme immunoassay (UniCAP system; Pharmacia & Upjohn Diagnostics). An IgE concentration exceeding 0.35 KIU/L was considered to represent specific sensitization.

HLA typing. Genomic DNA was extracted from peripheral blood leukocytes following standard phenol-chloroform method. Polymerase chain reaction (PCR) was performed in a total volume of 20 μ L. The PCR mixture contained 2 μ L of buffer; 25 pmol/L each of dATP, dCTP, dGTP, and dTTP; 200 pmol of each primer; the nucleotide sequences of primers used for amplification of *DQA1* and *DQB1* according to the reported sequences^{4,5}; 100 ng of template DNA; and 1 unit of *Taq* DNA polymerase (Promega, Madison, WI). Reaction mixtures containing human growth hormone-specific primers was used as control. The PCR primers were 5'-CAG TGC CTT CCC AAC CAT TCC CTT A-3' and 5'-ATC CAC TCA CGG ATT TCT GTT GTG TTT C-3'. The primer pair amplified a 439-base pair fragment of the human growth hormone.⁶ Amplification was performed in a PCT-200 thermal cycler. After preheating for 1 minute at 94° C, 35 amplification cycles were performed. Each amplification cycle was performed with denaturation at 94° C for 30 seconds (DNA denaturing), 58° C for *DQA1* and 60° C for *DQB1* for 50 seconds (primer annealing), and 72° C for 30 seconds (primer extension), followed by a final extension at 72° C for 5 minutes. Amplification of DNA was verified using 2% agarose gel electrophoresis and visualized with ethidium bromide staining. Interpreta-

tion and assignment of genotype were based on the pattern and length of PCR products according to the original protocols.⁴

Statistical Analyses

As previously described,⁷ *HLA-DQA1*, *DQB1* alleles were determined by direct counting. Allelic frequency (in percent) was calculated as follows: $GF = n/N \times 100$, where GF is genotype frequency, n is number of individuals positive for each genotype, and N is total number of individuals tested.

The allelic frequencies were compared using the χ^2 test or Fisher's exact test (2-sided) if any value in a 2×2 table less than 5. Odds ratio (OR) value was calculated as $OR = ad/bc$, where a and c denote the number of individuals positive for a certain genotype of patient and control groups, and b and c denote the number of individuals negative for a certain genotype of patient and control groups. An OR of greater than 1 means the gene carrier is susceptible to allergic rhinitis, and an OR of less than 1 means the gene carrier is not susceptible to allergic rhinitis. According to Bonferroni's rule,⁸ the corrected P values (*P_c*) were obtained by multiplying P values by the number of alleles tested. All comparisons were made in a 2-sided manner. The level of significance was set at 0.05. Analysis was done by using SPSS version 10.0 software.

RESULTS

Clinical Materials

Of the 41 patients with allergic rhinitis, all had obvious clinical symptom in autumn and showed positive results for the intracutaneous test of *Artemisia* pollen, all demonstrated a raised sIgE level against *Artemisia* pollen (as defined by an IgE level above 0.35 KIU/L), and 10 of them were sensitive to the same allergen as their directly related kin.

Distribution of *DQA1*, *DQB1* Allelic Frequencies in the Allergic Rhinitis and Control Groups

Distribution of *HLA-DQA1* allelic frequency among the allergic rhinitis patient and control groups. The allelic frequency of the *HLA-DQA1*0201* was significantly decreased (*P*

Table 1. Allelic frequency of *HLA-DQA1* gene in allergic rhinitis and controls

<i>HLA-DQA1</i> alleles	Patients (n = 41)		Controls (n = 41)		<i>P</i>	<i>P_c</i>
	No.	GF (%)	No.	GF (%)		
0101	2	4.88	4	9.76	NS	NS
0102	3	7.32	3	7.32	NS	NS
0103	5	12.20	9	21.95	NS	NS
0104	3	7.32	2	4.88	NS	NS
0201	10	24.39	19	46.34	0.038	0.38
0301	12	29.27	17	41.46	NS	NS
0302	24	58.54	6	14.63	0.00004	0.0004
0401	9	21.95	4	9.76	NS	NS
0501	12	29.27	16	39.02	NS	NS
0601	2	4.88	2	4.88	NS	NS

GF, Genotype frequency; *P_c*, *P* corrected for the number of alleles tested (n = 10).

= 0.038) in the patient group (24.39%) compared with the control subjects (46.34%). The OR value of patient group with respect to control subjects was 0.374 (95% confidence interval [CI], 0.014 to 0.957) (Table 1). The allelic frequency of the *HLA-DQA1*0302* was very significantly increased (*P* = 0.0004, *P_c* = 0.004) in the patient group (58.54%) compared with the control subjects (14.63%). The OR value of the patient group with respect to control subjects was 8.235 (95% CI, 1.837 to 23.909).

Distribution of *HLA-DQB1* allelic frequency among the allergic rhinitis patient and control groups. The allelic frequency of *HLA-DQB1*0602* was significantly decreased (*P* = 0.007) in the patient group (4.88%) compared with the control subjects (26.83%). The OR value of patient group with respect to control subjects was 0.140 (95% CI, 0.029 to 0.679) (Table 2).

DISCUSSION

Many investigations show that allergic rhinitis is a polygenic hereditary disease. Allergic rhinitis is a type I allergic disease and clusters within families. Twin studies have revealed that monozygotic twins are more concordant for atopic allergy than dizygotic twins.⁹ An adult with a family history of allergic rhinitis has a risk of 2- to 6-fold for developing rhinitis over adults without a family history.¹⁰ Because of various reasons, it is difficult to decipher the genetics of the allergic

Table 2. Allelic frequency of *HLA-DQB1* gene in allergic rhinitis and controls

<i>HLA-DQB1</i> alleles	Patients (n = 41)		Controls (n = 41)		<i>P</i>	<i>P_c</i>
	No.	GF (%)	No.	GF (%)		
0201	7	17.07	3	7.32	NS	NS
0301	1	2.44	5	12.20	NS	NS
0302	3	7.32	3	7.32	NS	NS
0303	11	26.83	5	12.20	NS	NS
0304	4	9.76	4	9.76	NS	NS
0401	7	17.07	6	14.63	NS	NS
0402	4	9.76	0	0	NS	NS
0501	13	31.71	15	36.59	NS	NS
0502	1	2.44	1	2.44	NS	NS
0503	1	2.44	2	4.88	NS	NS
0504	1	2.44	4	9.76	NS	NS
0601	10	24.39	4	9.76	NS	NS
0602	2	4.88	11	26.83	0.007	0.13
0603	5	12.20	4	9.76	NS	NS
0604	2	4.88	5	12.20	NS	NS
0605	6	14.63	5	12.20	NS	NS
0606	3	7.32	4	9.76	NS	NS
0607	0	0	1	2.44	NS	NS
0608	1	2.44	0	0	NS	NS

GF, Genotype frequency; *P_c*, *P* corrected for the number of alleles tested (n = 19).

disease.¹ More than 20 genes involved in the development of allergic diseases.² As one of the major genes, human MHC HLA class II regulates the human antigen-specific IgE responses.¹¹ The relationship between the HLA class II antigen and sensitization to various allergens has been researched in many areas. Because of the polymorphism of HLA genes and the genetic heterogeneity, conflicting results have resulted. Lara-Marquez et al¹² reported the association of mite-sensitive asthma to the haplotype *HLA-DRB1*1101*, *DQA1*0501*, *DQB1*0301* in the Venezuelan population. Torres-Galvan et al¹³ reported linkage of house dust mite allergy with *HLA-DRB1*, *-DQA1*, and *-DQB1* in the Spanish population. Donfack et al¹⁴ found that *HLA-DRB1*01* allele is associated with sensitization to cockroach allergens in the Hutterites. Many of the HLA genetics studies are about asthma and atopy. The genetics of rhinitis has not been studied as much as that of asthma and atopy. One of the principal reasons for this is the difficulty in the precise and discriminating “allergic rhinitis” phenotype characterization in a general population or in families and the fact that numerous rhinology disorders can show the same symptoms.³ In our

study, all of the patients must be in accord with the strict phenotype definition. Every patient must finish the strict questionnaires, nasal examination, and allergy tests. Everyone must have the typical chronic nasal obstruction or congestion, rhinorrhea, sneezing symptoms for at least 2 years in the autumn season, positive results of specific serum IgE, intracutaneous tests of *Artemisia* pollen, nasal mucosal scraping mast cell counting, or nasal discharge eosinophil counting. Through this method, perhaps we can prevent or alleviate the mixed factors from affecting the accuracy of the results.

Artemisia pollen has been considered one of the main causes of allergy in many different areas all over the world.¹⁵⁻¹⁷ It is also a major cause of intermittent allergic rhinitis in the northern area of China in the autumn. However, to our knowledge, there has been no other study to associate *Artemisia* pollen-induced allergic rhinitis with HLA class II genes. By means of PCR amplification with sequence-specific primers method, we have done research on association between the susceptibility of allergic rhinitis sensitization to *Artemisia* pollen and genotypes of *DRB1* in a previous study. We found that *HLA-DRB1*0301.2* and *DRB4*0101* might decrease the risk of developing

allergic rhinitis sensitization to *Artemisia* pollen.¹⁸ In the present study, we investigated the association between *HLA-DQA1* and *HLA-DQB1* loci with *Artemisia* pollen-sensitive allergic rhinitis. The results showed that *HLA-DQA1* and *-DQB1* might be involved in the development of *Artemisia* pollen-induced allergic rhinitis. The alleles frequencies of *HLA-DQA1*0201* and *DQB1*0602* were lower in patients with allergic rhinitis compared with the controls (24.39% versus 46.34%, $P = 0.038$, $P_c = 0.38$; 4.88% versus 26.83%, $P = 0.007$, $P_c = 0.013$); these alleles may be protective genes. The genotype frequencies of *DQA1*0302* were significantly increased among the allergic rhinitis group compared with the control group (58.54% versus 14.63%, $P = 0.00004$, $P_c = 0.0004$). The OR value was 8.235. Perhaps this allele is a susceptible gene. To the best of our knowledge, this is the first association study between *Artemisia* pollen-induced allergic rhinitis and *HLA-DQA1* and *-DQB1* genes.

The key of the study about the association between disease and gene lies in strictly selection of control group and elimination of latent individuals.¹² Although we eliminated the immunologic patients through strict use of questionnaire, in the control group we do not have all of the same experimental data as we have for the allergic rhinitis patients, which might influence the results.

In conclusion, our results shows the importance of the *HLA-DQA1* and *HLA-DQB1* genes in the development of allergic rhinitis sensitization to *Artemisia* pollen. *HLA-DQA1*0201* and *-DQB1*0602* alleles might confer protection against allergic rhinitis, and *DQA1*0302* may be a susceptible factor for hypersensitivity to *Artemisia* pollen. However, the development of rhinitis in *Artemisia* pollen-sensitive patients may also require the contribution from other candidate genes, controlling non-antigen-specific immune mechanisms such as STAT6 and IFN γ genes.¹⁹ Environmental factors also have important effects on inducing allergic rhinitis. It will be important in our future study to screen other susceptible genes and to study the relationship between the environmental and genetic factors in the susceptibility of allergic rhinitis.

The authors thank Prof De-Yun Wang (National University of Singapore) for critical review of the manuscript.

REFERENCES

1. Leung DY. Molecular basis of allergic diseases. *Mol Genet Metab* 1998;63:157-67.
2. Holgate ST. Asthma genetics: waiting to exhale. *Nat Genet* 1997;15:227-9.
3. Bousquet J, Van Cauwenberge P, Khaltaev N. Allergic rhinitis and its impact on asthma. *J Allergy Clin Immunol* 2001;108:S147-270.
4. Olerup O, Aldener A, Fogdell A. HLA-DQB1 and -DQA1 typing by PCR amplification with sequence-specific primers (PCR-SSP) in 2 hours. *Tiss Antigens* 1993; 41:119-34.
5. Picard JK. Single-step allele-specific polymerase chain reaction HLA-DQ genotyping using ARMS primers. *Hum Immunol* 1993;38:115-22.
6. Spriewald BM, Wassmuth R, Carl HD, et al. Microchimerism after liver transplantation: prevalence and methodological aspects of detection. *Transplantation* 1998;66: 77-83.
7. Cho SH, Kim YK, Oh HB, et al. Association of HLA-DRB1*07 and DRB1*04 to citrus red mite (*Panonychus citri*) and house dust mite sensitive asthma. *Clin Exp Allergy* 2000;30:1568-75.
8. Svejgaard A, Ryder LP. HLA and disease associations: detecting the strongest association. *Tiss Antigens* 1994; 43:18-27.
9. Duffy DL, Martin NG, Battistutt D, et al. Genetics of asthma and hay fever in Australian twins. *Am Rev Respir Dis* 1990;142:1351-8.
10. Lundback B. Epidemiology of rhinitis and asthma. *Clin Exp Allergy* 1998;28(suppl 2):3-10.
11. Shirakawa I, Deichmann KA, Izuhara I, et al. Atopy and asthma: genetic variants of IL-4 and IL-13 signalling. *Immunol Today* 2000;21:60-4.
12. Lara-Marquez ML, Yunis JJ, Layrisse Z, et al. Immunogenetics of atopic asthma: association of DRB1*1101 DQA1*0501 DQB1*0301 haplotype with *Dermatophagoides* spp. -sensitive asthma in a sample of the Venezuelan population. *Clin Exp Allergy* 1999;29:60-71.
13. Torres-Galvan MJ, Quirarte J, Blanco C, et al. Linkage of house dust mite allergy with the HLA region. *Ann Allergy Asthma Immunol* 1999;82:198-203.
14. Donfack J, Tsalenko A, Hoki DM. HLA-DRB1*01 alleles are associated with sensitization to cockroach allergens. *J Allergy Clin Immunol* 2000;105:960-6.
15. Katial RK, Lin FL, Stafford WW, et al. Mugwort and sage (*Artemisia*) pollen cross-reactivity: ELISA inhibition and immunoblot evaluation. *Ann Allergy Asthma Immunol* 1997;79:340-6.
16. Carinanos P, Galan C, Alcazar P, et al. Allergenic pollen in the subdesert areas of the Iberian Peninsula. *Invest Allergol Clin Immunol* 2000;10:242-7.
17. Park HS, Lee MK, Hong CS. Bronchial challenge responses in asthmatic patients sensitized to *Artemisia* spp pollen. *Yonsei Med J* 1989;30:173-9.
18. Xing Z, Yu D. Linkage of allergic rhinitis with HLA-DRB alleles polymorphism. *J Clin Otorhinolarygol (China)* 2001;15:199-201.
19. Barnes KC. Evidence for common genetic elements in allergic disease. *J Allergy Clin Immunol* 2000;106:S192-200.