

Linkage of house dust mite allergy with the HLA region

María-José Torres-Galván, ScD*; Joaquín Quiralte, MD†; Carlos Blanco, MD†; Rodolfo Castillo, MD†; Teresa Carrillo, MD†; Paloma Pérez-Aciego, PhD‡; and Florentino Sánchez-García, MD‡

Background: Atopy is a multifactorial disease, the pathogenesis of which is influenced by both genetic and environmental conditions. Genes in the HLA region have been involved in the control of the IgE response.

Objective: In order to investigate whether allergy to house dust mite is associated with HLA in our population, we performed sib-pair analysis in 18 families and a case/control study of 161 non-related individuals.

Methods: Levels of total and specific IgE were determined, skin-prick tests were carried out and clinical history was reviewed for every subject in the study. HLA class II typing was performed by the polymerase chain reaction with sequence specific primers.

Results: We observed a significant difference from expected values in haplotypes shared by affected sibs; however, the case/control study did not reveal any association with any particular allele.

Conclusion: These results suggest that any particular HLA-DRB1/DQA1/DQB1 allele is responsible for the development of allergy to house dust mite in the Spanish population. Some other locus in or close to the HLA region might be involved, eg, the tumour necrosis factor gene, a possibility that would explain the significant difference from expected values in the segregation of HLA haplotypes.

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INTRODUCTION

Atopy is considered to be a multifactorial disease, characterized by persistent immunoglobulin E (IgE) responses to ubiquitous aeroallergens. After initial sensitization in predisposed individuals, re-exposure to the allergen results in the IgE-mediated degranulation of mast cells, inflammation of airways, wheezing, and rhinitis.

Numerous studies of the general population and within families have suggested that both genetic and environmental influences are involved in the pathogenesis of asthma and atopy.^{1,2} Despite considerable evidence for heritability, genetic analysis has been difficult and results have been contradictory.

The antigen-specific IgE responses are restricted by the major histocompatibility complex (MHC). This region includes genes for human leucocyte antigen class II molecules (HLA-DR, -DQ and -DP), which are involved in the recognition and presentation of exogenous peptides. Many studies have attempted to identify an association between atopy and genetic markers in or near the MHC, but results have been inconclusive.^{3–6}

Positive skin tests to house dust mite are linked to a significant increase in risk for bronchial hyperresponsiveness and asthma, as compared with reactivity to grass pollens.^{7,8} Only the association between HLA-DR and a minor ragweed allergen (Amb a V) has been confirmed.^{9,10}

The population in the Canary Islands possess caucasoid genetic traits, similarly to those reported from other Spanish population.^{11,12} The geographic situation (a subtropical area at sea level) provide the adequate cli-

matic conditions (warm temperatures and a high relative humidity) for the growth of mites. In fact, the house dust mite (HDM) *Dermatophagoides pteronyssinus* (Der p) is the most prevalent allergen.^{13,14}

The present study was designed to investigate a possible genetic linkage between allergy to HDM and HLA, by means of affected sib-pair analysis, in 18 nuclear families. In addition, the possible HLA association with the atopic disease was examined in a case/control study, performed with a carefully selected group of atopic patients and non-atopic control subjects.

MATERIALS AND METHODS

Subjects

Eighteen nuclear families (n = 97) from Gran Canaria, Canary Islands, Spain, with two or more affected siblings, were recruited through probands at the Allergy Section of our hospital. Each proband had a history of rhinoconjunctivitis and/or bronchial asthma of at least 1 year's duration.

In addition, 76 unrelated atopic and 85 unrelated non-atopic individuals were selected and included in the case/control study.

Subjects were diagnosed as atopic if they met the 3 following criteria: (1) rhinoconjunctivitis and/or bronchial asthma; (2) positive skin-prick test to Der p; and (3) high IgE specific for Der p. Subjects negative to all three criteria were classified as non-atopic.

Informed consent was obtained from the subjects participating in the study, or from their parents. The protocol was approved by the Investigation and Ethics Committees of our hospital.

Skin Prick Tests

Skin prick tests (SPTs) were carried out in patients and control subjects by

* Research Unit, † Allergy Section and ‡ Immunology Unit; Hospital Nuestra Señora del Pino, Las Palmas de Gran Canaria, Canary Islands, Spain.

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a standard method.^{15,16} The following commercially available allergen extracts were used: *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Tyrophagus putrescentiae*, *Lepidoglyphus destructor*, *Alternaria alternata*, *Olea europaea*, *Lolium perenne*, *Cynodon dactylon*, *Parietaria judaica*, *Artemisia vulgaris*, *Plantago lanceolata*, dog dander and cat dander (100 BU/mL. Abelló S.A., Spain); *Aspergillus fumigatus*, *Cladosporium herbarum*, *Penicillium notatum* and rabbit epithelium (5% wt/vol, Laboratorio Ifidesa-Arístegui SA, Spain); and *Blatella germanica* (1 mg/mL. Laboratorio Ifidesa-Arístegui SA, Spain). Histamine phosphate (10 mg/mL) and physiologic saline solution were used as positive and negative controls, respectively. Tests were performed by puncture with a Morrow-Brown needle (Bayer, SA, Madrid, Spain) on the volar side of the forearms. After 15 minutes, a wheal with a diameter 3 mm greater than the saline control was considered a positive response.

IgE

Total serum concentrations of IgE were determined by the DELFIA method (Pharmacia Diagnostics, Uppsala, Sweden). A value of total serum IgE above 100 kU/L was considered as high for adults. For children (subjects of less than 15 years old), the value of cut off considered was contingent upon the age of the subject. The limits were set on the basis of the ranges of normality used by the laboratory of our hospital.

IgE specific to Der p was quantitated with the commercially available ImmunoCap allergens for the Pharmacia ImmunoCAP system, according to the manufacturer's instructions. Levels of or above 0.70 kU/L were considered to represent a positive response.

DNA Extraction

Genomic DNA from each subject was isolated from a whole blood sample by phenol/chloroform extraction and ethanol precipitation, according to a standard procedure.¹⁷

HLA Typing

HLA-DRB1, -DQA1 and -DQB1 typing was performed by sequence-specific primers PCR (PCR-SSP). The amplification of specific alleles or groups of alleles was carried out according to the manufacturer's instructions (Dynal AS, Oslo, Norway).

Assignment of alleles was based on the presence or absence of the amplified products, which were visualized by electrophoresis in 2.5% agarose gels, stained with ethidium bromide. One hundred and fourteen DRB1, 26 DQB1 and 14 DQA1 alleles were tested. These alleles were: DRB1*0101-04, *1501-04, *1601-06, *0301-04, *0401-19, *1101-19, *1201-03, *1301-24, *1401-17, *0701, *0801-11, *0901, *1001; DQA1*0101-05, *0201, *0301-03, *0401, *0501-03, *0601; and DQB1*0501-04, *0601-12, *0201-03, *0301-05, *0401-02.

A pair of primers that amplified the gene for human growth hormone was included in each reaction mix, as an internal positive control for amplification.

Statistical Analysis

Genetic linkage between allergy to HDM and HLA was investigated by the "affected sib-pair" method.¹⁸ The observed frequency for sharing 2, 1 or 0 HLA haplotypes among affected sib-pairs was compared with the expected pattern of normal haplotype segregation: 25%, 50%, and 25% (1:2:1) for sharing 2, 1 and 0 haplotypes, respectively. Genetic linkage is suggested if there is a significant difference between the observed and expected ratios of haplotypes segregation. Haplotype sharing among affected sib-pairs was only recognized if all four parental haplotypes were unambiguously identified, and sib-pairs from multiple sibships were treated as independent.

Frequencies of HLA genotypes were compared using the chi-squared test. The *P* values were corrected by multiplying them by the number of antigens tested (Bonferroni correction).¹⁹ When conditions were unsuitable for the chi-squared test, we used Fisher's exact test. A *P* value of less than .05 was

considered to indicate a statistically significant difference between groups.

RESULTS

Population Data

The 18 nuclear families consisted of 97 subjects: 56 males (52% atopic) and 41 females (58% atopic). The mean age and standard deviation was 14.8 ± 5.6 years for the sibs and 44.8 ± 6.9 years for the parents.

The group of unrelated subjects consisted of 76 atopic and 85 non-atopic individuals. Of these 161 subjects, 53 were male (46% atopic) and 108 were female (48% atopic). The mean age was 36.7 ± 13.6 (SD) years. Mean ages, standard deviations, and ranges were almost identical for the two sexes.

The contingency table of atopic status versus total IgE status showed a significant difference from the expected values ($\chi^2 = 59.0$, $P < .0001$).

Most patients (95%) were polysensitized, having positive results in SPTs with at least one different mite allergen than Der p (mainly, *D. farinae*). Some of them had also positive SPTs with other allergens, such as animal danders (49%), pollens (15%) and molds (5%).

Affected Sib-Pair Analysis

To evaluate possible genetic linkage between atopy and HLA haplotypes, we applied the sib-pair method of genetic analysis to several phenotypes: atopy, high total serum IgE (both as defined by us), asthma, and rhinoconjunctivitis. In each case, offsprings suffering from the corresponding condition were included in the analysis. The number of affected sibs was different in each case, data are shown in Table 1. We observed a significant increase over expected values in the number of HLA haplotypes shared by affected sibs when analysing the atopic status and total serum IgE levels.

HLA-Antigen Association

To investigate whether some HLA class II allele might be associated with atopic disease in our population, we performed a case/control study in our group of unrelated subjects. No signif-

icant difference in frequencies of HLA-DRB1, -DQA1, and -DQB1 alleles was found between patients and control subjects. Tables 2 and 3 show the data for the DRB1 and DQB1 loci (alleles in groups). Table 4 shows the allele level for DQA1 locus.

DISCUSSION

Atopy, the underlying cause of asthma and rhinitis, is a multifactorial condition, depending upon both genetic and environmental influences. It is known that a predisposition to allergic diseases is inherited, even though the mode of inheritance remains unclear. Several studies of a possible genetic linkage to atopy have failed to confirm a codominant, recessive, or dominant mode of inheritance.²⁰ Other studies have suggested a dominant pattern^{21,22} or a recessive one.^{9,23} It is obviously difficult that the results of genetic studies fit into a mendelian pattern of inheritance with such a polygenic background.

Given the key role of HLA molecules in the regulation of the immune response, genetic epidemiologic studies have attempted to relate responsiveness to specific allergens to HLA type. HLA class II genes of the MHC, encoded on chromosome 6p, have been implicated in the susceptibility to a number of diseases that are known to have an immunologic basis.²⁴ Previous

Table 1. Results of "Affected Sib-Pair" Analysis

Phenotype	Sib-Pairs*	Shared Haplotypes			χ^2	P
		2	1	0		
Atopy†	32	15	11	6	8.2	<.02
High serum IgE†	47	20	22	5	9.8	<.01
Asthma	16	5	7	4	0.4	NS‡
Rhinoconjunctivitis	21	9	7	5	0.4	NS

* Number of affected sib-pairs.

† As defined in text.

‡ NS, not significant.

studies have yielded conflicting results about an association between atopy and HLA.^{5,6} In the atopic disease, only an association between the minor ragweed allergen Amb a V and HLA-DR2 has been consistently confirmed.²⁵⁻²⁷ Some other reported associations are Lol p I, Lol p II, Lol p III (rye grass), and DRB1*03 and DRB1*11^{28,29}; Ole e I (olive pollen) and DRB1*07 and DQB1*0201³⁰; Bet v I (birch pollen) and DRB3*0101³¹; and Chi t I (non biting midge) and DRB1*0101, DQA1*0101, DQB1*0501.³²

It must be taken into account that the definition of the allergic phenotype presents a great variation among studies, with the common feature of a clinical history of atopy. In many cases, fulfilling only one additional criterium is enough to establish the atopic status (eg, an enhanced total serum IgE, a positive SPT or RAST score with any of the allergens tested).

In our study, we performed complete HLA-DRB1, -DQA1, and -DQB1 typing by PCR-SSP, a high-resolution DNA-based method that allows an accurate detection of HLA class II polymorphism and, therefore, a more precise identification of a possible association with specific IgE responses.

In order to establish whether a particular HLA allele is associated with atopic disease in our population, we performed a case/control study. The prevalence of atopy has been reported to be 43% in the population of the Canary Islands.¹³ Thus, random sampling of healthy controls is inadequate, since the association of genetic factors with disease might be masked. All our control subjects were screened for the three criteria that were established for identification of "atopic individuals": a clinical history of rhinoconjunctivitis and/or asthma; positive results of SPT with Der p; and a serum level of Der p-specific IgE of or above 0.70 kU/L. Negative responses allowed a true comparison between atopic and non-atopic individuals.

We did not find any evidence of an association between allergy to Der p and a particular HLA-DR/DQ allele. Nevertheless, our sib-pair analysis revealed a significant increase in haplotypes shared by atopic sibs, as compared with the expected values. This finding supports the existence of some "atopic factor" in the MHC region. Likewise, when the considered phenotype was high total serum IgE, the sib-pair analysis showed a positive result, confirming the implication of this region in the control of the atopic response.

Table 2. Distribution of HLA-DRB1 Antigens in the Study Population

DRB1	Patients (n = 76)		Controls (n = 85)		P	Pc
	n	%	n	%		
01	14	18.4	21	25.0	.09	NS
15	5	6.6	16	19.0	.01	NS
16	8	10.5	5	6.0	.13	NS
03	17	22.4	15	17.9	.12	NS
04	13	17.1	24	28.6	.03	NS
11	26	34.2	17	20.2	.02	NS
12	2	2.6	0	0.0	.24	NS
13	26	34.2	29	34.5	.13	NS
14	0	0.0	2	2.4	.30	NS
07	20	26.3	24	28.6	.13	NS
08	5	6.6	2	2.4	.14	NS
09	2	2.6	1	1.2	.39	NS
10	3	3.9	3	3.6	.33	NS

NS, not significant; Pc, corrected P value.

Table 3. Distribution of HLA-DQB1 Antigens in the Study Population

DQB1	Patients (n = 76)		Controls (n = 85)		P	Pc
	n	%	n	%		
05	28	36.8	31	36.9	.13	NS
06	25	32.9	35	41.7	.07	NS
02	31	40.8	34	40.5	.13	NS
07	31	40.8	28	33.3	.08	NS
08	13	17.1	15	17.9	.16	NS
09	3	3.9	6	7.1	.19	NS
04	4	5.3	0	0.0	.05	NS

NS, not significant; Pc, corrected *P* value.

Table 4. Distribution of HLA-DQA1 Alleles in the Study Population

DQA1	Patients (n = 76)		Controls (n = 85)		P	Pc
	n	%	n	%		
0101	15	19.7	21	25.0	.11	NS
0102	29	38.2	31	36.9	.13	NS
0103	8	10.5	14	16.7	.10	NS
0104	0	0.0	2	2.4	.30	NS
0105	3	3.9	3	3.6	.32	NS
0201	19	25.0	24	28.6	.12	NS
0301	12	15.8	9	10.7	.12	NS
0302	0	0.0	1	1.2	.57	NS
0303	5	6.6	12	14.3	.06	NS
0401	4	5.3	0	0.0	.05	NS
0501	42	55.3	37	44.0	.05	NS
0503	1	1.3	1	1.2	.57	NS

NS, not significant; Pc, corrected *P* value.

A first explanation of our results might come from the fact that crude extracts were used for SPTs instead of purified allergens, what happens in most of the studies. Complex antigens such as those of Der p express multiple epitopes that can be presented by different HLA class II molecules on the surface of antigen-presenting cells. This phenomenon limits the possibility of finding any association between particular HLA alleles and specific IgE responses to a given allergen. Nevertheless, a work made with highly purified allergens⁴ revealed no excess of any HLA-DR/DP allele in subjects responding to the group I and II allergens of *Dermatophagoides pteronyssinus* (Der p I and Der p II), when compared with non-responder individuals.

Several reports have showed, in studies of particular HDM-atopic individuals, that Der p I and Der p II

contain several overlapping T-cell epitopes restricted by different HLA class II molecules.^{33,34} A different restriction pattern has been obtained investigating cloned T lymphocytes induced with *Dermatophagoides* spp.^{35,36} illustrating the heterogeneity of the restriction specificity of these allergens. Furthermore, a recent study was not able to demonstrate significant differences in gene frequencies of HLA class II alleles in Der p I-sensitized children.³⁷

All the previous studies corroborate the difficulty of establishing particular associations between HDM allergy and HLA class II alleles. At the moment, there are no conclusive results that can be reproduced in different populations, even in Europe. Many factors might be acting, as the considered atopic phenotype, the complexity of Der p aller-

gens, and cross-reactivity with *D. farinae*.

It is likely that other loci are relevant in determining an individual's susceptibility to specific allergens. Regarding the HLA region, a possible candidate is the tumour necrosis factor (TNF) gene, a potent pro-inflammatory cytokine that is found in increased concentration in asthmatic airways³⁸ and in lavage fluid from asthmatic lungs.³⁹ A recent study shows that genotypes known to correlate with increased TNF secretion are associated with an increased risk of asthma.⁴⁰

Several loci have been implicated in the control of the level of the atopic immune response. Linkage analysis on chromosome 11q12-13 have suggested that the gene of the β -chain of the high-affinity IgE receptor (Fc ϵ RI- β) is linked to atopy⁴¹⁻⁴³; however, other study has not confirmed this result.⁴⁴ The interleukin 13/4/5/GM-CSF cluster on chromosome 5q31 has been also shown to be linked to total levels of IgE and bronchial hyperresponsiveness.⁴⁵⁻⁴⁷ An ongoing study is being performed in our population to try to expand these findings.

In summary, allergy to HDM is a multifactorial condition that arises from complex interactions between genetic and environmental factors. Genes related to susceptibility, located either within or outside the HLA region (TNF, IL-4, Fc ϵ RI- β , etc), might play an important role in the development of atopic disease.

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- Request for reprints should be addressed to:*
María-José Torres-Galván
Unidad de Investigación
Hospital Nuestra Señora de Pino
Ángel Guimerá, 93
35004 Las Palmas de Gran Canaria
Spain
-