

Genetic basis of the latex-fruit syndrome: Association with HLA class II alleles in a Spanish population

Carlos Blanco, MD, PhD,^a Florentino Sánchez-García, MD, PhD,^b María-José Torres-Galván, PhD,^c Antonio G. Dumpierrez, MD,^a Lourdes Almeida, MD,^a Javier Figueroa, MD,^a Nancy Ortega, MD,^a Rodolfo Castillo, MD, PhD,^a María-Dolores Gallego, MD,^a and Teresa Carrillo, MD, PhD^a Canary Islands, Spain

Background: The latex-fruit syndrome is a well-defined disorder whose genetic background has not been elucidated. **Objective:** To study the genetic basis of the latex-fruit syndrome.

Methods: In a case-control study, we have investigated a carefully selected group of patients allergic to latex, searching for association between latex-fruit allergy and HLA class I and II genes, HLA-DR functional groups, and markers *IL4-RI* and *FcεRI-βca*.

Results: Seventy-eight patients allergic to latex without spina bifida, 33% of them also allergic to fruits, were included in our protocol. Skin prick test results with both a commercial latex extract and purified hevein were significantly greater in patients allergic to latex and fruit than in patients allergic to latex and not fruit. A cutoff point of >7 mm for commercial latex skin prick test diagnosed latex-fruit allergy with a sensitivity of 66.7% (95% CI, 41.0-86.6) and a specificity of 83.3% (95% CI, 68.6-93.0) in our series of patients. No significant differences were found regarding HLA class I, *IL4-RI*, or *FcεRI-βca* allele distributions. However, comparison of HLA class II allelic frequencies between patients allergic to latex and fruit and patients allergic to latex and not fruit showed significant associations of latex-fruit allergy with DQB1*0201 (corrected *P* value, .001; odds ratio, 7.3; 95% CI, 2.6-20.0), as well as with HLA-DR functional group E (corrected *P* value, .028; odds ratio, 16.0; 95% CI, 1.9-134.1). When comparing allelic distribution among different subgroups of patients allergic to latex, additional significant associations of latex-fruit allergy with DRB1*0301 and *0901, and of latex and not fruit allergy with DQB1*0202, DRB1*0701 and *1101, were demonstrated.

Conclusions: Latex-fruit allergy is associated with HLA-DQB1*0201, DRB1*0301, and *0901, as well as with HLA-DR functional group E, whereas latex-not-fruit allergy is associated with DQB1*0202, and with both DRB1*0701 and *1101 alleles. (J Allergy Clin Immunol 2004;114:1070-6.)

Key words: Latex allergy, latex-fruit syndrome, hevein, HLA, microsatellite

Natural rubber latex IgE-mediated hypersensitivity is probably one of the most important health problems we have faced in allergy during recent years.^{1,2} There are several reasons for this, including a high prevalence in certain populations at risk, frequent severe reactions—especially during surgery and medical procedures—and its ability to induce occupational asthma among workers using gloves. Another argument for great concern is that latex allergy is significantly associated with certain food allergies, the so-called *latex-fruit syndrome*, which has clear clinical consequences.^{3,4} Recent investigations point to a group of defense-related plant proteins, class I chitinases, as the panallergens responsible for latex-fruit allergy.⁵ These enzymes have a N-terminal hevein-like domain, which shows IgE cross-reactivity with latex hevein, or Hev b 6.02, a major latex allergen.⁶

Candidate gene approach and genome-wide searches have been used to identify chromosomal regions relevant to allergy and related phenotypes, such as asthma, atopy, and IgE levels.⁷ Among the most promising linkages, there is a locus in chromosome 6, corresponding to the HLA region, the chromosome 5 cytokine gene cluster, which includes IL-4 gene, and another locus in chromosome 11, containing the gene for the β-chain of the high affinity receptor for IgE (FcεRI-β).⁸ Products of these genes play a critical role in allergic reactions regarding antigen presentation, IgE synthesis, and activation of mast cells, respectively.

In this context, allergic responses to antigens such as ragweed Amb a 1⁹ and rye grass Lol p 1 and 2¹⁰ have been linked with specific MHC class II genes. Recent data suggest that grouping HLA-DR alleles into functional categories regarding subregion structures within the peptide binding groove may help when studying associations of HLA with disease.¹¹ Interestingly, *IL4-RI*, located in the second intron of the IL-4 gene (chromosome 5q31-q33), has shown strong linkage to total IgE levels in an inbred population.¹² In addition, *FcεRI-βca*, located in the fifth intron of the FcεRI-β gene (chromosome 11q13), has shown linkage to atopy, asthma, and total IgE in several populations.¹³

There have been a few previous studies concerning genetics of latex allergy. HLA class II antigen DQ8 alone,

From ^aSección de Alergia, ^bServicio de Inmunología, and ^cUnidad de Investigación, Hospital de Gran Canaria Dr Negrín.

Partially supported by Instituto de Salud Carlos III (Fondo de Investigaciones Sanitarias grant 00/1019, ISCiii-RTIC-G03/094, and RedRespira-ISCiii-RTIC-C03/11), and by Fundación de la Sociedad Española de Alergología e Inmunología Clínica.

Received for publication February 16, 2004; revised June 2, 2004; accepted for publication June 4, 2004.

Reprint requests: Carlos Blanco, MD, PhD, Servicio de Alergia, Hospital de Gran Canaria Dr Negrín, C/Bco de la Ballena s/n, 35012 Las Palmas de GC, Spain. E-mail: cblague@gobiernodecanarias.org.

0091-6749/\$30.00

© 2004 American Academy of Allergy, Asthma and Immunology
doi:10.1016/j.jaci.2004.06.022

Abbreviations used

OR: Odds ratio
Pc: Corrected *P* value
ROC: Receiver operating characteristic
SPT: Skin prick test
SSO: Sequence-specific oligonucleotide

the HLA-DQ8-DR4 haplotype, or both, seem to be associated with allergy to hevein in health care workers.^{14,15} No association has been found in patients with spina bifida and latex allergy with or without sensitization to Hev b 1.¹⁶ However, these studies have not taken latex-fruit allergy into consideration. Moreover, they have neither searched for possible association with *IL4-R1* and *FcεRI-βca* markers nor evaluated HLA-DR allele functional groups. The current protocol was designed to investigate further the genetic background of latex allergy, focusing on the latex-fruit syndrome.

METHODS

Selection of patients and control subjects

A prospective study was designed in our allergy section, composed of a detailed clinical history, *in vivo* skin tests, and serum extraction for *in vitro* assays. Patients reporting immediate adverse reactions in relation to latex exposure, suggestive of being IgE-mediated, and showing a positive skin prick test (SPT) to a latex commercial extract (ALK-Abelló, Madrid, Spain) were included in our protocol.

For statistical analysis, patients allergic to latex patients were further stratified in different subgroups according to presence or absence of atopy, food hypersensitivity—see definitions below—occupational exposure, or severity of latex adverse reactions. For this purpose, patients with latex-induced systemic anaphylaxis or bronchial asthma were classified as having severe latex allergy, whereas patients with milder symptoms were classified as having not severe latex allergy.

A group of control subjects matched for age, sex, and atopy with our patients was also recruited. The same *in vivo* and *in vitro* tests were performed in both patients and control subjects. Written informed consent was obtained from patients and controls, and the protocol of this study was approved by the Research and Ethics Committees of the Hospital.

SPTs

Skin prick tests were performed in all patients and controls by trained personnel in a blind manner, according to standard procedure, with a panel of aeroallergen commercial extracts (ALK-Abelló): dust mites (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Tyrophagus putrescentiae*, and *Lepidoglyphus destructor*), molds (*Aspergillus fumigatus*, *Cladosporium herbarum*, and *Alternaria alternata*), pollens (*Olea europaea*, *Lolium perenne*, *Artemisia vulgaris*), and animal epithelia (cat, dog, rabbit, and horse). Atopy was diagnosed on the basis of a clinical history of respiratory allergy and positive SPT to at least one of these common aeroallergens.

Patients were also tested with a panel of food extracts, including banana (ALK-Abelló), chestnut, kiwi (Bial-Aristegui, Bilbao, Spain),

and an avocado extract made in our laboratory.¹⁷ If other foods were implicated in adverse reactions by a given patient, or if there was discordance between clinical history and SPT with the commercial food extract, prick by prick tests with fresh foods were also performed. Food immediate hypersensitivity was diagnosed on the basis of a suggestive clinical history, complemented with a positive skin test to the corresponding food.

Skin prick tests were performed in a subgroup of patients with purified hevein (kindly provided by Z. Chen, BGFA, Bochum, Germany),¹⁸ diluted in PBS from a lyophilized sample with added glycerin (50% vol/vol) to final protein concentrations of both 1 and 10 µg/mL, and stored at 4°C until used.

Histamine dihydrochloride (10 mg/mL) and physiologic saline solution served as positive and negative controls, respectively. The SPT was considered positive if the mean wheal diameter was greater than 3 mm 15 minutes after puncture compared with the negative control.

Total and specific IgE determination

Total IgE was determined by the ImmunoCAP System (Pharmacia Diagnostic, Uppsala, Sweden), following the manufacturer's instructions. Specific IgE to latex (code k82) and to the foods implicated in adverse reactions for each patient were also determined with the CAP, following the manufacturer's recommendations, and values greater than 0.35 kU/L were considered positive.

DNA extraction

Genomic DNA was isolated from 10 mL EDTA-anticoagulated peripheral blood samples by phenol-chloroform extraction and ethanol precipitation, according to a standard procedure.¹⁹ DNA samples were used for both HLA and microsatellite typing.

HLA typing

HLA-A, HLA-B, and HLA-Cw typing was performed by a PCR with sequence-specific oligonucleotide (SSO) commercial method (Dynal Rely SSO-A, B, C, Dynal Biotech Ltd, Bromborough, United Kingdom). HLA-DRB1, DQB1 and DPB1 typing was performed by another PCR-SSO standard technique (InnoLipa-DRB1, -DQB and -DPB, Innogenetics, Gent, Belgium).²⁰ Briefly, aliquots of genomic DNA were subjected to PCR amplification with appropriate gene primers, then hybridized with membranes preloaded with group-specific probes, and the resulting band patterns interpreted to assign alleles presented in each HLA locus. HLA specificities analyzed were those defined by the World Health Organization HLA Nomenclature Committee until 2001.²¹

HLA-DRB1 and DQB1 high-resolution typing

HLA-DRB1 and DQB1 high-resolution typing was performed by a PCR with sequence-specific primer commercial method (Dynal AllSet + SSP, Dynal Biotech).²² PCR amplification of genomic DNA, with specific pairs of primers for each allele, was performed according to the manufacturer's instructions. Assignment of alleles was based on the absence or presence of the amplified products, which were visualized by electrophoresis in 2.5% agarose gels, stained with ethidium bromide, and photographed under UV light. Again, HLA-DRB1 and DQB1 specificities analyzed were those defined by the World Health Organization HLA Nomenclature Committee until 2001.²¹

Microsatellite typing

Previously described primers were used for microsatellite typing.^{23,24} HEX (IL4-R1) and 6-FAM (FcεRI-β)-labeled primers (*) were provided by Applied Biosystems (Foster City, Calif) with the following sequences:

TABLE I. Clinical features of patients and control subjects

	Patients with latex allergy			Controls
	Latex-not-fruit	Latex-fruit	Total	
No. subjects	52	26	78	68
Mean age $\pm$ SD	34.4 $\pm$ 13.1	36.8 $\pm$ 9.8	35.2 $\pm$ 12.2	36.1 $\pm$ 8.3
Sex (F/M)	44/8	25/1	69/9	61/7
Atopic disease (%)	59.6	61.5	60.3	58.8
Occupational allergy (%)	59.6	69.2	62.8	0
Latex SPT (mean $\pm$ SD)	5.8 $\pm$ 2.4*	8.3 $\pm$ 2.9*	6.6 $\pm$ 2.8	0
Total IgE†	180.2 (146.7)	147.2 (151.6)	169.3 (148.3)	189.5 (152.6)
Latex specific IgE†	2.2 (224.6)	3.5 (163.6)	2.5 (205.8)	0

*Latex SPT mean wheal diameter showed a significant difference ($P < .001$) between patients with latex-fruit and latex-not-fruit allergy.

†Expressed as geometric mean in kU/L (coefficient of variation).

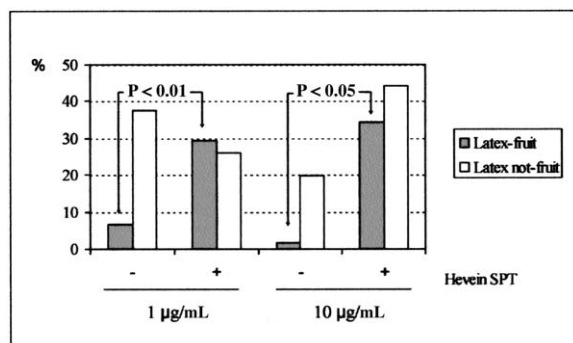


FIG 1. Relation between hevein SPT and concomitant fruit allergy in a group of 61 patients with latex allergy. Results are expressed as a percentage of patients with latex-fruit or latex-not-fruit allergy showing negative (–) or positive (+) hevein SPT, tested at 2 different concentrations (1 and 10 µg/mL). A significant association between hevein SPT results and latex-fruit allergy was demonstrated.

IL4-R1a* 5'TGCACCTGGGCAACAGTTTA
IL4-R1b 5'GTTGGATGGACTTGGAGATT
FceRI-βa* 5'ATCTACTGCAAGTGACGATC
FceRI-βb 5'CATCTCCCTACCATCTGACC

Microsatellite typing was performed by multiplex PCR on a GeneAmp PCR System 9600 thermocycler (Applied Biosystems). Reactions were performed in a final volume of 15 µL, with genomic DNA (50 ng), 1.5 mmol/L MgCl₂, dNTPs (200 µmol/L each), primers (5 pmol each), and 0.5 U EcoTaq polymerase (Ecogen, Barcelona, Spain). The amplification procedure was as follows: 5 minutes at 94°C; 25 cycles of 30 seconds at 94°C, 30 seconds at 64°C, and 30 seconds at 72°C; and 15 minutes at 72°C.

The PCR samples (1 µL) were added to 0.2 µL ROX-labeled GeneScan-500 size standard (Applied Biosystems) and 20 µL formamide, denatured (2 minutes at 95°C), and rapidly chilled on ice. Samples were analyzed by capillary electrophoresis in an ABI PRISM 310 Genetic Analyzer (Applied Biosystems).

Statistical analysis

Allelic and genotypic frequencies were analyzed by the gene counting method. Evaluation of frequency distributions was made by the χ^2 or Fisher exact tests, as appropriate. For multiple allelic frequency comparisons, P values were corrected (P_c) by multiplying them by the number of alleles tested (Bonferroni correction).²⁵ A P or

P_c value of less than .05 was considered to indicate a statistically significant difference between groups. The area under the receiver operating characteristic (ROC) curve, sensitivity, specificity, odds ratio (OR), and 95% CI, were calculated by standard methods, when needed.

RESULTS

Patients and controls

Seventy-eight patients were included in our study because they had a compelling history of latex IgE-mediated allergy and showed positive latex SPT. Their clinical characteristics are summarized in Table I. They were 69 women (88.5%) and 9 men, with a mean age ($\pm$ SD) of 35.2 $\pm$ 12.2 years, with only 6 of them younger than 18 years. They had no spina bifida or urogenital abnormalities, although 3 of them had undergone multiple surgical procedures.

Forty-seven patients (60.3%) were atopic—most of them ($n = 45$) showing dust mite respiratory allergy, with or without other aeroallergen sensitizations—49 (62.8%) were exposed to latex in an occupational setting, and 26 of them (33.3%) were diagnosed of concomitant fruit allergy. Sixty-eight control subjects, with no history of latex allergy and showing negative latex SPT, were also recruited. They were 61 women (89.7%) and 7 men, whose age (mean $\pm$ SD, 36.1 $\pm$ 8.3 years) and atopy rate (58.8% with respiratory allergy to dust mites) did not significantly differ from our patients'.

Results of SPT and IgE determinations

Skin prick test and IgE determination results are also summarized in Table I. SPT with the latex commercial extract was positive in all patients, with a mean wheal diameter ($\pm$ SD) of 6.6 $\pm$ 2.8 mm. Patients with latex-fruit allergy showed significantly greater latex SPT compared with patients with latex-not-fruit allergy (8.3 mm $\pm$ 2.9 mm vs 5.8 mm $\pm$ 2.4 mm; $P < .001$). Total serum IgE geometric mean (range) was 169.3 kU/L (18–2000) in our patients, and it was <100 kU/L in 29 (37.2%) of them. Latex specific IgE geometric mean was 2.5 kU/L, and latex specific IgE determinations yielded negative results in 12 patients (15.4%). No differences were found in total

TABLE II. Area under the ROC curves and best cutoff values for diagnosing fruit allergy in a group of patients with latex allergy, as estimated for a latex commercial extract and purified hevein SPT

Extract	Area under the ROC curve (95% CI)	Best cutoff value		
		Criterion*	Sensitivity (95% CI)	Specificity (95% CI)
Latex commercial	0.808 (0.686-0.898)	>7	66.7% (41.0-86.6)	83.3% (68.6-93.0)
Hevein 1 µg/mL	0.846 (0.731-0.926)	>2	90% (68.3-98.5)	61% (44.5-75.8)
Hevein 10 µg/mL	0.812 (0.691-0.900)	>6	70% (45.7-88.0)	78% (62.4-89.4)

*Expressed as millimeters of SPT mean wheal diameter.

TABLE III. Frequencies (in percentages of patients) of HLA-DQ alleles

DQ allele	Patients with latex allergy			Controls (n = 68)
	Latex-not-fruit (n = 52)	Latex-fruit (n = 26)	Total (n = 78)	
DQB1*0201	5.8†	30.8†	14.1	14.0
DQB1*0202	17.3‡	1.9‡	12.2	14.0
DQB1*0301	16.3	11.5	14.7	14.7
DQB1*0302	18.3	23.1	19.9	14.0
DQB1*0303	0.0	0.0	0.0	2.2
DQB1*0304	0.0	1.9	0.6	0.7
DQB1*0305	0.0	1.9	0.6	0.7
DQB1*0402	1.9	3.8	2.6	0.7
DQB1*0501	13.5	5.8	10.9	10.3
DQB1*0502	1.9	1.9	1.9	1.5
DQB1*0503	0.0	1.9	0.6	2.2
DQB1*0601	0.0	0.0	0.0	0.7
DQB1*0602	6.7	3.8	5.8	7.4
DQB1*0603	9.6	5.8	8.3	7.4
DQB1*0604	7.7	3.8	6.4	8.1
DQB1*0606	0.0	1.9	0.6	0.7
DQB1*0609	1.0	0.0	0.6	0.7

† $P < .001$; P corrected value = .001; OR, 7.3; 95% CI, 2.6-20.0.

‡ $P = .006$; P corrected value, nonsignificant.

IgE or specific IgE to latex between patients with latex-not-fruit allergy and latex-fruit allergy. All control subjects showed negative specific IgE to latex.

Skin prick tests with purified hevein, tested at both 1 and 10 µg/mL, were performed in 61 of the 78 patients allergic to latex, showing positive results in 55.7% and 78.7% of them, with a mean wheal diameter ($\pm$ SD) of 3.0 mm $\pm$ 2.4 mm and 5.5 mm $\pm$ 3.7 mm, respectively. As shown in Fig 1, a positive SPT to hevein used at a concentration of 1 µg/mL was significantly associated with concomitant fruit allergy ($P = .002$; OR, 6.5; 95% CI, 1.8-22.7). Similarly, when hevein was used at a concentration of 10 µg/mL, a positive result was also associated with the presence of fruit allergy in patients with latex allergy ($P = .016$; OR, 9.3; 95% CI, 1.1-77.6).

The area under the ROC curves and best cutoff values for diagnosing latex-fruit allergy are shown in Table II, as estimated for latex commercial and hevein SPT. In this context, only 4 of 27 patients (14.8%) with negative SPT to hevein at 1 µg/mL showed concomitant fruit allergy; meanwhile, only 1 of 13 patients (7.7%) with negative SPT to hevein at 10 µg/mL did. On the contrary, the

TABLE IV. Frequencies (in percentages of patients) of HLA-DRB1 functional groups

Group	Patients allergic to latex			Controls (n = 68)
	Latex-not-fruit (n = 52)	Latex-fruit (n = 26)	Total (n = 78)	
A	20.2	21.2	20.5	19.1
De	28.8	23.1	26.9	22.8
Dr	19.2	15.4	17.9	14.7
E	1.0*	13.5*	5.1	7.4
Q	17.3†	3.8†	12.8	14.7
R	5.8†	19.2†	10.3	13.2
a	7.7	3.8	6.4	8.1

* $P = .004$; P corrected value = 0.028; OR, 16.0; 95% CI, 1.9-134.1.

† $P = .02$; P corrected value, nonsignificant.

proportion of patients with latex allergy with associated fruit allergy rose to 43.7% or 52.9% when only patients with positive hevein SPT, tested at either 10 or 1 µg/mL, respectively, were considered.

HLA class I and II genes

Analysis of HLA class I -A, -B and -Cw allelic frequencies showed no significant differences between patients with latex allergy and controls, and both populations were in Hardy-Weinberg equilibrium for every gene studied. Similarly, when comparing HLA class I typing results among the different subgroups of patients with latex allergy, no significant differences were found.

In the same way, HLA class II DRB1, DQB1 and DPB1 allelic frequencies showed no significant differences between patients and controls. However, comparison of HLA-DQB1 allelic frequencies between patients with latex-fruit allergy and latex-not-fruit allergy showed a significant association of DQB1*0201 with latex-fruit allergy ($P = .001$; OR, 7.3; 95% CI, 2.6-20.0; Table III). A trend toward an increase in DQB1*0202 frequency in patients with latex-not-fruit allergy, compared with patients with latex-fruit allergy, was also observed ($P = .09$).

HLA-DRB1 functional groups

HLA-DR allelic frequencies were reanalyzed by grouping them into 7 different functional categories, attending to the structure of the pocket 4 in the peptide binding groove (Table IV).¹¹ Again, no differences were found between patients with latex allergy and controls, but

TABLE V. Summary of significant allelic associations found when comparing allelic distribution among different subgroups of patients allergic to latex (latex-fruit vs latex-not-fruit, and positive vs negative hevein SPT at 1 μ g/mL)

Associated to	Allele	P value	OR (95% CI)
Latex-fruit	DQB1*0201	<.001	14.0 (3.9-50.3)
	DRB1*0301	.003	6.3 (1.7-23.3)
	DRB1*0901	.012	3.2 (2.3-4.6)
	DRB1-FGE†	.002	15.3 (1.7-135.2)
Latex-not-fruit	DQB1*0202	.003	13.2 (1.6-105.8)
	DRB1*0701	.01	6.3 (1.3-30.0)
	DRB1*1101	.05	1.5 (1.3-1.9)
Positive hevein SPT at 1 μ g/mL	DRB1-FGE†	.022	2.0 (1.5-2.5)

†HLA-DRB1 functional group E.

patients with latex-fruit allergy showed a significantly higher frequency of functional group E than patients with latex-not-fruit allergy ($P = .028$; OR, 16.0; 95% CI, 1.9-134.1). A trend was observed toward an association of latex-fruit allergy with functional group R, as well as of latex-not-fruit allergy with functional group Q, but P values were not significant after Bonferroni correction in either case.

Comparisons of HLA class II allelic and functional group distributions

Further statistical analysis was performed by assessing allelic distribution among the different subgroups of patients, ie, by comparing the presence or absence of a specific allele in given subgroups of patients.

These results are summarized in Table V, demonstrating additional significant associations of latex-fruit allergy with DRB1*0301 and *0901. Latex-not-fruit allergy was significantly related to DQB1*0202, as well as to DRB1*0701 and *1101. HLA-DR functional group E showed significant associations not only with latex-fruit allergy but also with a positive hevein SPT tested at 1 μ g/mL.

IL4-R1 and Fc ϵ RI- β ca markers

Regarding *IL4-R1*, we identified 16 alleles ranging from 150 to 180 bp. *Fc ϵ RI- β ca* analysis showed 9 alleles ranging from 114 to 132 bp. All PCR amplifications yielded detectable products. In both cases, the ranges of allele size were similar to those of other studies. Allelic frequencies of *IL4-R1* and *Fc ϵ RI- β ca* markers did not show significant differences either between patients and control subjects or when considering the various subgroups of patients allergic to latex.

DISCUSSION

Because both prevalence and severity of allergic diseases are increasing in industrialized countries, much interest has been focused in prevention. An understanding

of the genetic bases for allergic conditions is essential, not only to explain their pathophysiology but also to prevent them. It is now well known that allergy and asthma are complex diseases, which means that the risk of developing such phenotypes depends on the interaction among diverse polymorphic genes, each exerting a small effect, and environmental stimuli.^{7,8} Thus, elucidating the genetic background of such complex diseases is rather difficult. Approaches like family studies or segregation analysis have been used to investigate them.

With regard to latex allergy, its low prevalence in the general population as well as the lack of a single test with proper diagnostic accuracy limit such studies.^{1,2} Therefore, we chose to perform a case-control study on a carefully selected population of patients with latex allergy, to search for significant associations with certain polymorphic genes known to be linked to allergy and related phenotypes by previous investigations. In particular, HLA genes as well as *IL4-R1* and *Fc ϵ RI- β ca* markers were our candidates, although we were conscious that several other genes may be involved.

We also took into consideration that latex major allergen hevein is the kind of small but powerful allergen,¹⁸ probably with few relevant epitopes regarding antigen presentation,²⁶ in which associations with specific MHC class II loci could be expected. In this context, previous studies on the genetics of latex allergy had managed to demonstrate a significant association between allergy to hevein in health care workers and HLA class II antigen DQ8 alone, the HLA-DQ8-DR4 haplotype, or both.^{14,15}

Latex-fruit syndrome is a well-defined disorder affecting from 20% to 60% of patients with latex allergy, depending on the study considered.^{3,4} Although other allergens may be involved, it seems that cross-reactivity of the N-terminal hevein-like domain of class I chitinases with latex major allergen hevein is responsible for most latex-fruit-associated allergies.⁵ This fact can explain why we have found a significant relation between hevein SPT results and latex-fruit allergy. Moreover, according to our data, a cutoff point can be established for hevein SPT to predict latex-fruit allergy, with acceptable sensitivity and specificity. This could have clinical implications on daily practice once a commercial hevein extract for SPT becomes available. At the same time, this higher sensitization to hevein in patients with latex-fruit allergy compared with patients with latex-not-fruit allergy, may account for the significantly greater SPT wheal diameters that we have observed in subjects with latex-fruit allergy when using a commercial latex extract. Thus, in our series of patients, a commercial latex SPT result higher than a cutoff point of 7 mm diagnosed latex-fruit allergy with a sensitivity of 66.7% and a specificity of 83.3%. Of course, these cutoff values need to be verified with additional independent data sets.

Not surprisingly, we have not been able to demonstrate any significant differences between patients with latex allergy and controls either in HLA class I and II or in *IL4-R1* and *Fc ϵ RI- β ca* alleles. Moreover, we could not confirm a previously reported association between hevein

sensitization and HLA class II antigens,^{14,15} probably because of different selection criteria and our smaller sample size. Nevertheless, comparison between patients with latex-fruit allergy and latex-not-fruit allergy has allowed us to describe several significant associations not previously reported of various HLA class II alleles with either latex-fruit or latex-not-fruit allergies. Specifically, latex-fruit allergy showed association to HLA-DQB1*0201, DRB1*0301, and *0901, whereas latex-not-fruit allergy showed association to DQB1*0202, DRB1*0701, and *1101 alleles.

Because both groups of alleles are known to be in a disequilibrium linkage, our results suggest the existence of 2 different HLA haplotypes associated, respectively, with latex-fruit and latex-not-fruit allergy in our population, although they could also point to another gene or genes linked to the HLA region. Given the role of HLA class II molecules in antigen recognition and presentation, our data reveal a difference in the ability of individuals with latex-fruit and latex-not-fruit allergy to respond to antigens involved in these reactions. Thus, subjects bearing DRB1*0301, *0901, and DQB1*0201 molecules would be able to respond to latex-fruit common epitopes, whereas the carriers of DRB1*0701, *1101, and DQB1*0202 molecules would recognize different epitopes present only in latex. Joining these findings with our previous studies on the hevein-like domain of plant class I chitinases,^{5,6} we can postulate that subjects bearing DRB1*0301, *0901, and DQB1*0201 molecules may respond to the cross-reactive peptides of the hevein-like domain that have been recently identified.²⁷

Many previous studies had reported associations of some of these HLA alleles with a variety of autoimmune processes. In fact, both DQB1*0201 and DRB1*0301 alleles, which increased risk for latex-fruit allergy in our population, are part of the ancestral haplotype 8.1 that predisposes white populations to an impressive number of autoimmune diseases.²⁸ In contrast, the extended DRB1*0701, DQB1*0202 haplotype, which we have identified as protective against fruit allergy in patients with latex allergy, had been found to be associated with the lack of antibody response to hepatitis B vaccination²⁹ and to protect against certain autoimmune diseases.³⁰

However, studies connecting these HLA alleles with allergy and related phenotypes are scarce. Specifically, DQB1*0201 allele has shown association with high levels of specific IgE to olive tree pollen antigens Ole e 1 and Ole e 3,³¹ as well as to susceptibility to isocyanate-induced asthma.³² DQB1*0202 has been related to nasal polyposis,³³ DRB1*0301 to poor response to grass pollen specific immunotherapy,³⁴ and DRB1*1101 to mite-sensitive bronchial asthma,³⁵ as well as to high levels of specific IgE against *Parietaria* major allergen Par o 1.³⁶ More interestingly, the high frequency of DRB1*0701 (DR7) phenotype among patients with spina bifida with latex or hevein responsiveness,¹⁶ together with the protective effect that—according to our results—DRB1*0701 seems to exert against latex-fruit allergy, could further explain the lower prevalence of latex-fruit allergy in

patients with latex allergy and spina bifida compared with patients without spina bifida.

Regarding the analysis of the HLA-DR functional groups, we had previously reported a significant association of the Dr group with dust mite allergy, thus suggesting that grouping HLA-DR alleles into functional categories may assist in the search for predictive factors in atopic disease.³⁷ Our suggestion is now reinforced by the finding of a significant association between latex allergy and HLA-DR allele functional group E. Because this functional group includes DRB1*0901,¹¹ this result corresponds with what we could have expected.

In conclusion, we have described a possible genetic basis of the latex-fruit syndrome, including HLA class II alleles for both susceptibility and protection. Our findings could help explain the pathophysiology of the latex-fruit syndrome and could have future applications in the field of primary prevention of latex-fruit allergy in health care workers.

We thank Zhiping Chen for supplying purified hevein; Rosabel Afonso and Juan Blanco for technical assistance; and all of the personnel from the Allergy Section of Hospital Dr Negrín for their invaluable collaboration.

REFERENCES

1. Poley GE Jr, Slater JE. Latex allergy. *J Allergy Clin Immunol* 2000;105:1054-62.
2. Charous BL, Blanco C, Tarlo S, Hamilton RG, Baur X, Beezhold D, et al. Natural rubber latex allergy after 12 years: recommendations and perspectives. *J Allergy Clin Immunol* 2002;109:31-4.
3. Blanco C, Carrillo T, Castillo R, Quirarte J, Cuevas M. Latex allergy: clinical features and cross-reactivity with fruits. *Ann Allergy* 1994;73:309-14.
4. Blanco C. Latex-fruit syndrome. *Curr Allergy Asthma Rep* 2003;3:47-53.
5. Blanco C, Diaz-Perales A, Collada C, Sanchez-Monge R, Aragoncillo C, Castillo R, et al. Class I chitinases as potential panallergens involved in the latex-fruit syndrome. *J Allergy Clin Immunol* 1999;103:507-13.
6. Diaz-Perales A, Sanchez-Monge R, Blanco C, Lombardero M, Carrillo T, Salcedo G. What is the role of the hevein-like domain of fruit class I chitinases in their allergenic capacity? *Clin Exp Allergy* 2002;32:448-54.
7. Steinke JW, Borish L, Rosenwasser LJ. Genetics of hypersensitivity. *J Allergy Clin Immunol* 2003;111(suppl):S495-501.
8. Borish L. Genetics of allergy and asthma. *Ann Allergy Asthma Immunol* 1999;82:413-24.
9. Marsh DG, Hsu SH, Roebber M, Ehrlich-Kautzky E, Freidhoff LR, Meyers DA, et al. HLA-Dw2: a genetic marker for human immune response to short ragweed pollen allergen Ra5, I: response resulting primarily from natural antigenic exposure. *J Exp Med* 1982;155:1439-51.
10. Freidhoff LR, Ehrlich-Kautzky E, Meyers DA, Ansari AA, Bias WB, Marsh DG. Association of HLA-DR3 with human immune response to Lol p I and Lol p II allergens in allergic subjects. *Tissue Ant* 1988;31:211-9.
11. Ou D, Mitchell LA, Tingle AJ. A new categorization of HLA DR alleles on a functional basis. *Hum Immunol* 1998;59:665-76.
12. Marsh DG, Neely JD, Breazeale DR, Ghosh B, Freidhoff LR, Ehrlich-Kautzky E, et al. Linkage analysis of IL4 and other chromosome 5q31.1 markers and total serum immunoglobulin E concentrations. *Science* 1994;264:1152-6.
13. Folster-Holst R, Moises HW, Yang L, Fritsch W, Weissenbach J, Christophers E. Linkage between atopy and the IgE high-affinity receptor gene at 11q13 in atopic dermatitis families. *Hum Genet* 1998;102:236-9.
14. Rihs HP, Chen Z, Cremer R, Baur X. HLA class II antigens DR4 and DQ8 are associated with allergy to hevein, a major allergen of *Hevea latex*. *Tissue Ant* 1997;49:92-5.

15. Rihs HP, Chen Z, Rueff F, Cremer R, Raulf-Heimsoth M, Baur X, et al. HLA-DQ8 and the HLA-DQ8-DR4 haplotype are positively associated with the hevein-specific IgE immune response in health care workers with latex allergy. *J Allergy Clin Immunol* 2002;110:507-14.
16. Rihs HP, Cremer R, Chen Z, Baur X. Molecular analysis of DRB and DQB1 alleles in German spina bifida patients with and without IgE responsiveness to the latex major allergen Hev b 1. *Clin Exp Allergy* 1998;28:175-80.
17. Blanco C, Carrillo T, Castillo R, Quirarte J, Cuevas M. Avocado hypersensitivity. *Allergy* 1994;49:454-9.
18. Chen Z, Posch A, Lohaus C, Raulf-Heimsoth M, Meyer HE, Baur X. Isolation and identification of hevein as a major IgE-binding polypeptide in Hevea latex. *J Allergy Clin Immunol* 1997;99:402-9.
19. Blin N, Stafford DW. A general method for isolation of high molecular weight DNA from eukaryotes. *Nucleic Acids Res* 1976;3:2303-8.
20. Buyse I, Decorte R, Baens M, Cuppens H, Semana G, Emonds MP, et al. Rapid DNA typing of class II HLA antigens using the polymerase chain reaction and reverse dot blot hybridization. *Tissue Ant* 1993;41:1-14.
21. Marsh SG. WHO Nomenclature Committee for Factors of the HLA System. Nomenclature for factors of the HLA system, update December 2001. *Tissue Ant* 2002;59:172-3.
22. Olerup O, Zetterquist H. HLA-DR typing by PCR amplification with sequence-specific primers (PCR-SSP) in 2 hours: an alternative to serological DR typing in clinical practice including donor-recipient matching in cadaveric transplantation. *Tissue Ant* 1992;39:225-35.
23. Marsh DG, Neely JD, Breazeale DR, Ghosh B, Freidhoff LR, Ehrlich-Kautzky E, et al. Linkage analysis of IL4 and other chromosome 5q31.1 markers and total serum immunoglobulin E concentrations. *Science* 1994;264:1152-6.
24. Sandford AJ, Shirakawa T, Moffatt MF, Daniels SE, Ra C, Faux JA, et al. Localisation of atopy and beta subunit of high-affinity IgE receptor (Fc epsilon RI) on chromosome 11q. *Lancet* 1993;341:332-4.
25. Dunn OJ. Multiple comparisons among means. *J Am Stat Assoc* 1961;56:52-64.
26. Beezhold DH, Kostyal DA, Sussman GL. IgE epitope analysis of the hevein preprotein; a major latex allergen. *Clin Exp Immunol* 1997;108:114-21.
27. Diaz-Perales A, Blanco C, Sanchez-Monge R, Varela J, Carrillo T, Salcedo G. Analysis of avocado allergen (Prs a 1) IgE-binding peptides generated by simulated gastric fluid digestion. *J Allergy Clin Immunol* 2003;112:1002-7.
28. Candore G, Lio D, Colonna Romano G, Caruso C. Pathogenesis of autoimmune diseases associated with 8.1 ancestral haplotype: effect of multiple gene interactions. *Autoimmun Rev* 2002;1:29-35.
29. McDermott AB, Zuckerman JN, Sabin CA, Marsh SG, Madrigal JA. Contribution of human leukocyte antigens to the antibody response to hepatitis B vaccination. *Tissue Ant* 1997;50:8-14.
30. Drouet M, Delpuget-Bertin N, Vaillant L, Chauchaix S, Boulanger MD, Bonnetblanc JM, et al. HLA-DRB1 and HLA-DQB1 genes in susceptibility and resistance to cicatricial pemphigoid in French Caucasians. *Eur J Dermatol* 1998;8:330-3.
31. Cardaba B, Cortegano I, Florido F, Arrieta I, Aceituno E, del Pozo V, et al. Genetic restrictions in olive pollen allergy. *J Allergy Clin Immunol* 2000;105:292-8.
32. Bignon JS, Aron Y, Ju LY, Kopferschmitt MC, Garnier R, Mapp C, et al. HLA class II alleles in isocyanate-induced asthma. *Am J Respir Crit Care Med* 1994;149:71-5.
33. Molnar-Gabor E, Endreffy E, Rozsasi A. HLA-DRB1, -DQA1, and -DQB1 genotypes in patients with nasal polyposis. *Laryngoscope* 2000;110:422-5.
34. Fleva A, Daniilidis M, Sidiropoulos J, Adam K, Tourkantonis A, Daniilidis J, et al. Can HLA typing predict the outcome of grass pollen immunotherapy?. *Exp Clin Immunogenet* 2001;18:13-23.
35. Lara-Marquez ML, Yunis JJ, Layrisse Z, Ortega F, Carvallo-Gil E, Montagnani S, 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.
36. D'Amato M, Picardi A, Menna T, Di Somma C, Ariano R, di Pietro A, et al. HLA-DRB1* and allergy to Parietaria: linkage and association analyses. *Hum Immunol* 1999;60:1250-8.
37. Torres-Galvan MJ, Quirarte J, Blanco C, Castillo R, Carrillo T, Perez-Aciego P, et al. Pocket 4 in the HLA-DRB1 antigen-binding groove: an association with atopy. *Allergy* 2000;55:398-401.

Bound volumes available to subscribers

Bound volumes of *The Journal of Allergy and Clinical Immunology* are available to subscribers (only) for the 2004 issues from the Publisher, at a cost of \$115.00 for domestic, and \$137.00 for international subscribers for Vol. 113 (January-June) and Vol. 114 (July-December). Shipping charges are included. Each bound volume contains subject and author indexes. The binding is durable buckram with the journal name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact Elsevier Inc, Subscription Customer Service, 6277 Sea Harbor Dr, Orlando, FL 32887; phone (800) 654-2452 or (407) 345-4000.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular journal subscription.