

Genetics and specific immune response in allergy to birch pollen and food: Evidence of a strong, positive association between atopy and the HLA class II allele HLA-DR7

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Background: In some geographic areas birch pollen represents the most prominent cause for airborne allergic diseases. Up to 70% of patients allergic to birch pollen are hypersensitive to fruits, especially apples. Associations have been found, in some instances, with a sensitivity to aeroallergens and HLA class II genes.

Objectives: We investigated whether susceptibility or resistance to birch pollen allergy with and without food allergy was associated with HLA class II genes.

Methods: Blood samples were obtained from 2 groups of unrelated European-born white adults: 42 atopic patients (31 of them with asthma) and 42 healthy control subjects with no personal or familial history of asthma or atopy. Their antibody responses to birch pollen, apples, grass, and weed pollens were evaluated by skin tests, RASTs, and immunoprints. Genomic DNA was extracted from PBLs. The exons of *DQA1*, *DQB1*, *DRB1*, and *DPB1* genes were selectively amplified by using the PCR method. Genotyping was carried out by digestion of the amplified DNA products with allele-specific endonucleases (PCR-RFLP), which recognize allelic variations in the polymorphic exon.

Results: We found no significant differences in the frequency of *DPB1* alleles between patients and control subjects. HLA class II DR4 and/or DR7 alleles were present in 42.6% of the patients and in only 2.4% of the healthy subjects. These results confirm a previous study of a group of polysensitized atopic patients, which showed that DR4 and DR7 alleles were rare in healthy control subjects and frequently observed in atopic subjects with or without concomitant asthma.

Conclusion: We conclude that the allele HLA-DR7 is significantly involved in the presentation of apple and pollen allergens. However, we suggest that this susceptibility is more relat-

ed to atopy than to specific responses to allergens. (*J Allergy Clin Immunol* 1999;104:395-401.)

Key words: Atopy, birch pollen, food, specific immune responses, HLA class II genes

Previously, we have shown that some HLA class II alleles may provide protection against isocyanate-induced asthma and that isocyanate-induced asthma and extrinsic asthma probably differ in terms of their association with HLA class II genes.¹ Moreover, in a study of a large and extensive French family of 3 generations comprising 35 members, we reported that susceptibility to allergy, with or without asthma-related symptoms, was frequently associated with HLA class II-DR4 and/or DR7 specificities.² More recently,³ we observed in 2 groups of unrelated European-born white adults (both allergic patients and healthy asymptomatic control subjects) that HLA class II-DR4 and DR7 alleles were strongly associated with atopy. However, because the majority of patients involved in the latter study were polysensitized, the nature of the specific responses to given aeroallergens could not be determined.

HLA class II genes coding for human leukocyte antigen class II molecules are likely candidates for controlling the immune response to common allergens.⁴ Indeed, different HLA products or genes may represent risk factors for, or protective factors against, the development of asthma, which may or may not be associated with sensitization to allergens.^{4,5} Analyzing the results of previous investigations, only the association between the minor ragweed allergen Amb t 5 has been confirmed.^{6,7}

Indeed, the success of association studies critically depends on the appropriate selection of control subjects. The lack of a strong association between allergic diseases and HLA class II genes, as observed in previous studies, could be due to the high prevalence (about 30%) of atopy in control subjects who have been selected at random from the general population.

This study was designed to investigate whether the genetic association described previously³ could be confirmed in a group of allergic patients from a different geographic area who were tested for responses to partic-

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Abbreviation used

IEF: Isoelectric focusing

ular allergens, especially birch pollen and apple allergens. We also sought to determine whether significant correlations could be found between the latter HLA class II alleles and the specific reactions to any of the allergens. To test these hypotheses, we studied the distribution of haplotypes and alleles of the different HLA class II genes in 2 groups of unrelated white European subjects: (1) patients from eastern France, with or without asthma, who were all sensitized to birch pollen and (2) healthy asymptomatic control subjects. In view of the importance of phenotype characterization in case/control studies, a careful selection of nonatopic control subjects was carried out.

Our results confirm that a strong association exists between some HLA class II alleles and the susceptibility to atopy.

METHODS

This study was conducted in accordance with the principles of the Declaration of Helsinki, with informed consent and institutional approval.

Subjects

All subjects were European-born, unrelated white adults living in eastern France in the Strasbourg area. Forty-two patients (22 women and 20 men; mean age, 32.3 years) were admitted to the study, which was conducted in the Department of Pneumology, Strasbourg Civil Hospital. They were given a standardized questionnaire by physicians with extensive experience in allergic diseases.

All patients had documented atopic allergy (rhinitis, conjunctivitis, and respiratory complaints), with or without asthma-related symptoms, and had a positive skin prick test response to a mixed *Betulaeae* pollen extract (Stallergènes Laboratories, Fresnes, France). Phenotype included medical history and typical asthma symptoms (shortness of breath, wheezing, coughing, and chest tightness). Subjects were defined as atopic if they had positive test results for one or more of the following criteria.

- (1) Positive skin prick test responses as defined by a wheal of at least 75% of the mean diameter of that induced by a histamine control and carried out with a panel of common allergens, including grass and weed pollens (one negative control test with saline solution and one positive control test with histamine [1 mg/mL] were also applied);
- (2) a positive skin prick test response to a birch pollen extract (Stallergènes Laboratories) was required as a necessary criterion for birch sensitization;
- (3) a positive value for serum-specific IgE by RAST (Pharmacia); and
- (4) detection of specific IgE antibodies by immunoblotting techniques.

Total IgE was determined in 25 patients by paper radioimmunosorbent test (Pharmacia).

We also studied 42 control subjects originating either from the same geographic area or from the Paris area (28 women and 14 men; mean age, 41.5 years). Diagnosis of allergy was only based on clinical history in 21 control subjects. The control subjects answered the same questionnaire as the patient group. Prevalence of allergic

symptoms or atopy-related conditions among their first-degree relatives was also thoroughly investigated. Criteria for admission to the study included the absence of any personal and/or familial history of atopy through 3 generations. However, skin tests were also performed on 15 control volunteers who agreed to have them done in accordance with local ethical principles. RAST and specific IgE determination by immunoblotting was also performed on 6 control sera.

All individuals were included in the study only if they had no history of cancer, diabetes, cardiovascular disease, or other major disease.

Experimental protocol

Blood samples (20 mL) were obtained from all individuals. Serum samples for immunologic studies were separated by centrifugation and stored at 4°C in the presence of sodium azide (0.02%) until use. Genomic DNA was extracted from the peripheral blood leukocytes¹ or from jugal epithelial cells, as previously described.²

Immunologic studies. For allergenic extract, 280 mg of birch and 50 mg of *Dactylis glomerata* pollen (Allergon, Engelhom, Sweden) were incubated on a rotating drum for 1 hour at room temperature, with either 1.2 mL or 0.45 mL of sterile water, and then centrifuged at 4°C for 10 minutes at 13,000g. Total protein extraction from Golden Delicious apples (176 g) was performed with cold acetone. The different proteins (160 mg) were solubilized with 3.2 mL of 4.5 mol/L urea, 1% 3-[(3-cholamidopropyl)-dimethylammonio]-1-propane sulfonate (CHAPS, Sigma), and 1% 1,4-dithio-DL-threitol (Fluka) solution. The 3 different supernatants were used immediately.

Isoelectric focusing (IEF) was performed in a 0.8% agarose gel (Isogel, FMC) containing 12% sorbitol and 5% (vol/vol) of a mixture of carrier ampholytes (Pharmacia) to make a gradient gel ranging from pH 3.5 to pH 9.5.⁸ Apple protein separation was carried out in the presence of 4.5 mol/L urea and 1% CHAPS inside the gel. Extracts from birch pollen (80 µL), *Dactylis glomerata* (120 µL), and apple (275 µL) were loaded on the anode side. After IEF, one part of the gel was stained with Coomassie blue. Two pressure prints⁹ were removed from another part of the gel 10 seconds and 10 minutes after running and loaded onto a CNBr-activated nitrocellulose filter (Schleicher and Schuell, Dassel, Germany).¹⁰ After printing, the nitrocellulose was cut into 2.5-mm strips to be used for immunodetection.

For SDS-PAGE, samples of the allergenic extract were diluted twice with 76 mmol/L Tris-acetate buffer (pH 6.8), containing 2% SDS and bromophenol blue as a marker dye; they were incubated for 10 minutes at 60°C and applied to a 12% acrylamide gel (0.5 mm). A stacking gel containing 6% acrylamide was layered on top. Electrophoresis was carried out for 120 minutes at 250 V on a flatbed apparatus, cooled at 15°C (Multiphor II; Pharmacia Biotech, Uppsala, Sweden).¹¹

After the run, proteins were electrotransferred onto a CNBr-activated nitrocellulose membrane by using the semi-dry electroblotting technique (Pharmacia Biotech, Uppsala, Sweden) for 60 minutes at 1 mA/cm² with two buffers: 20 mmol/L Tris, 150 mmol/L glycine, and 20% ethanol on the anode side; 20 mmol/L Tris and 150 mmol/L glycine on the cathode side.

After the blocking step, all blot-activated nitrocellulose strips were incubated overnight, as previously described,¹² with patient's sera diluted 10-fold at room temperature in the blocking solution. After washing several times, IgE antibodies were detected by alkaline phosphatase-conjugated human anti-IgE goat antibodies at a dilution of 1:700 (Sigma, France).

The protein assay was performed by using the Micro BCA protein kit, according to the manufacturer's instructions (Pierce).

HLA typing. The DNA samples were typed for HLA class II

TABLE I. Number of patients with positive or negative responses to birch pollen allergens, with or without allergen association

Allergens	Clinical symptoms		Skin prick test responses		RAST results		IEF	
	+	-	+	-	+	-	+	-
Birch	42	0	42	0	41	0	42	0
Birch + apple	32	10	29	6	15	17	34	8
Birch + food*	40	2	19	11	14	14	ND	ND
Birch + grass	19	23	24	18	21	7	26	16
Birch + weeds	3	39	11	31	11	13	ND	ND
Birch + pets	2	40	14	28	12	8	ND	ND
Birch + mites	2	40	9	33	8	13	ND	ND

The allergens tested by IEF and causing positive results in patients were *Betula* and *Dactylis* pollens and apple extracts.

Total number of patients = 42.

ND, Not determined.

*Food = vegetables and fruits except apples.

TABLE II. Distribution of HLA-DRB1 alleles between the patient and control groups

Alleles	Patient group (n = 42)		Control group (n = 42)		Statistical tests	
	n*	%	n	%	χ^2 †	Corrected P values‡
DR1	3	7.1	6	14.3	0.50	4.8
DR2	15	35.7	18	42.8	0.45	5.0
DR3	7	16.6	8	19.0	0.08	8
DR4	9	21.4	1	2.4	5.56	.18
DR5	11	26.2	13	30.9	0.23	6.3
DR6	16	38.0	18	42.8	0.20	6.6
DR7	11	26.2	0	0	12.6	3.7×10^{-3}
DR8	1	2.4	1	2.4	0	10
DR9	3	7.1	0	0	1.38	2.9
DR10	0	0	6	14.3	4.49	3

*Number of phenotypes carrying the allele.

†Chi-square test with Yates correction if needed.

‡Corrected for the number of alleles tested (n = 10).

DRB, DQA1, DQB1, and DPB1 alleles and haplotypes by using the PCR-RFLP method, as reported in a previous work.¹

Statistical analysis

Phenotypic frequencies of HLA alleles were compared between groups by either the chi-square test (with Yates correction if needed) or the Fisher exact test with 2×2 contingency tables. The corrected *P* values were obtained by multiplying *P* values by the number of alleles tested.¹³ The level of significance was set at .05.

RESULTS

Clinical and functional data

Of the 42 patients, 31 had allergic asthma, and the remaining 11 had allergic rhinitis and/or conjunctivitis without asthma-associated symptoms. All 42 subjects had a documented history of symptoms related to at least birch pollen allergy (Table I). Thirty-two of them had clinical symptoms of apple allergy, and 34 recognized apple allergens as detected by immunoblotting techniques. Nineteen of 42 patients were sensitive to grass pollen, as shown by clinical symptoms, and 26 of them were found to react with grass allergens by IEF. Approximately 14 to 19 subjects who were sensitive to birch pollen had positive skin prick test responses and/or

RAST results to some fruits (ie, peach, hazelnut, walnut, and kiwi, but not apples) and to some vegetables (ie, celery and carrot). However, as seen in Table I, no good correlation was observed in the latter case between patient complaints and skin prick test and/or RAST results. Few individuals demonstrated reactivity to pet or mite allergens.

Total serum IgE was measured in 25 patients, of which only 11 demonstrated a raised total IgE level (>200 IU/mL), as defined by an IgE level 2 SD above the predicted mean for the age of the subject.¹⁴

As stated above, skin prick tests were performed on healthy volunteers only. However, in all subjects on whom the tests were performed (15 individuals), the test responses were negative. No detectable amounts of specific IgE were observed in the 6 nonallergic control sera, which were tested by RAST and immunoblotting techniques.

HLA typing

Distribution of DRB1 alleles. The distribution of DRB1 alleles among the patients and the control subjects is shown in Table II. The frequency of the HLA-DR7 alleles was very significantly increased in the patients (*P*

TABLE III. Distribution of DQA1 alleles between the patient and control groups

DQA1	Patient group (n = 42)		Control group (n = 40)		Statistical tests	
	n*	%	n	%	χ^2	Corrected <i>P</i> values†
0101, 0102‡	21	50.0	24	60.0	0.83	2.52
0103	14	33.3	12	30.0	0.11	5.25
0201	11	26.2	2	5.0	6.90	.063
0301	10	23.8	3	7.5	4.09	.28
0401	0	0	4	10.0	2.52	.77
0501	23	54.8	25	62.5	0.51	3.29
0601	1	2.4	0	0	—	7.0

*Number of phenotypes carrying the allele.

†Corrected for the number of alleles tested (n = 7).

‡Alleles DQA1*0101 and *0102 could not be differentiated.

TABLE IV. Distribution of DQB1 alleles between the patient and control groups

DQB1	Patient group (n = 42)		Control group (n = 39)		Statistical tests	
	n*	%	n	%	χ^2	Corrected <i>P</i> values†
0201	17	40.5	9	23.1	2.81	1.08
0301	15	35.7	11	28.2	0.52	5.64
0302	8	19.0	3	7.7	1.36	2.88
0303	4	9.5	0	0	2.14	1.68
0401, 0402‡	0	0	0	0	—	12.0
0501	3	7.1	10	25.6	5.14	.24
0502	1	2.4	6	15.4	2.84	1.08
0503	3	7.1	3	7.7	0.11	8.88
0601	1	2.4	0	0	0.0	11.64
0602, 0603‡	23	54.8	14	35.9	2.90	1.08
0604	2	4.8	6	15.4	1.51	2.64
0605	0	0	3	7.7	1.54	2.52

*Number of phenotypes carrying the allele.

†Corrected for the number of alleles tested (n = 12).

‡Alleles DQB1*0401 and *0402 and DQB1*0602 and *0603 could not be differentiated.

= 3.7×10^{-4}). The frequency of subjects carrying HLA-DR4 alleles was higher ($P = .018$) among the patient group (21.4%) than among the control subjects (2.4%); however, the difference was not significant when the P value was corrected for the number of alleles tested. When the frequency of the affected subjects carrying either DR4 or DR7 alleles or sharing both specificities (20 of 42 patients) was compared with that of the healthy control subjects (1 of 42 control subjects), the difference was highly significant ($P = .001$; corrected $P = .01$). No significant differences were noted between frequencies of DR4 subsets in the patient group, in which alleles DRB1*0401, *0402, *0403, *0404, and *0408 were prevalent.

There was no difference in the distribution of DRB alleles associated with DR3, DR5, DR6, and DR8 specificities in either group (results not shown).

Distribution of DQA1 alleles. A significant increase in alleles DQA1*0201 ($P = .009$) and DQA1*0301 ($P = .04$) was apparent in the patients compared with the control subjects; however, this difference did not reach statistical significance when P values were corrected for the number of alleles tested (Table III).

Distribution of DQB1 alleles. As shown in Table IV, the frequency of the allele DQB1*0501 showed a tendency to increase ($P = .02$) in the control individuals

compared with the patients; however, the difference was no more significant when P values were corrected for the number of alleles tested.

Distribution of DPB1 alleles. No significant difference was found in the distribution of DPB1 alleles between patients and control subjects (results not shown).

Comparison of HLA-DR4 and/or DR7 allele frequency in atopic patients with and without asthma-related symptoms

In allergic, nonasthmatic patients (11 of 42) 6 subjects carried either DR4 or DR7 alleles (54%) compared with 14 subjects (45%) in the group of allergic patients with asthma (31 of 42). This difference was not significant ($P = .85$).

Specific immunologic responses to allergens associated with HLA class II DR4 and DR7 alleles

Proteins from *Betula* species, *Dactylis* species, and apple extracts were separated in a wide pH range. After printing, the samples incubated with sera from 42 patients sensitive to birch pollen displayed different allergens (Fig 1 and Table V). The major allergen Bet v 1 (isoelectric point, 5.0 to 5.6) was recognized by 41 sera.

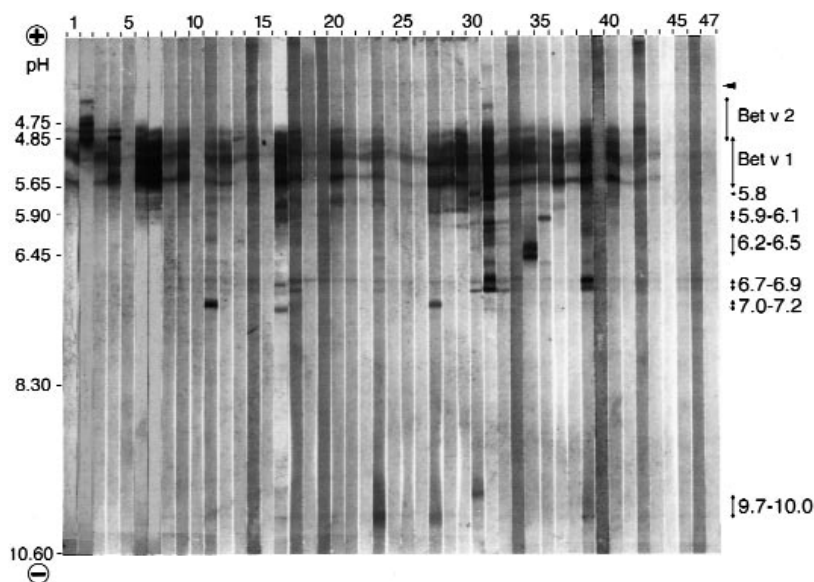


FIG 1. *Betula verrucosa*-binding patterns with IgE from patients allergic to birch pollen after IEF separation and printing. Lanes 1 to 9 and 11 to 43, incubation with individual sera of birch pollen-sensitive patients; lanes 44 to 47, sera of nonallergic patients; lane 10, buffer control. Left, Isoelectric points of protein markers (BDH); right, arrow indicating the deposit site. Known allergens are indicated by name, and unknown allergens are indicated by their isoelectric point range.

Five other allergens were revealed after IEF separation and immunoprinting (Table V). They were visualized with only a few patients' sera. However, 40% of the patients tested ($n = 17$) displayed reactivity to the allergen migrating to an isoelectric point of 6.7 to 6.9. Three patients' sera (Fig 1, rows 5, 15, and 39) did not recognize any birch allergen after IEF separation. However, after SDS-PAGE separation and Western blotting (data not shown), these sera exhibited Bet v 1 reactivity. With the latter separation method, the same allergens (molecular masses of 70 kd, 32 kd, 14 kd, and 12 kd) were visualized, and Bet v 1 was recognized by 97.6% of the patients' sera, as described in a previous work.¹⁵ Only 2 patients were found to display IgE reactivity against Bet v 2 (ie, profilin; Fig 1, lanes 2 and 31). As seen in Table V, about 81% of the patients are sensitive to the major apple allergen Mal d 1, and among them, 57% are sensitive to the Dac g 1 allergen (one of the major allergens of *Dactylis glomerata*).

We further investigated whether a correlation could be observed between the reactivity of the patients' sera to allergens other than Bet v 1 and the number of subjects carrying DR4 and/or DR7 alleles or DR7 alleles only. The patients with associated allergies were taken into account when clinical history, skin test reactivity, and immunoblotting results were in good correlation. However, when the number of patients sensitive to an additional allergen (ie, apple or grasses) and also carrying the latter alleles was compared with the number reacting to birch pollen only (Table VI), the difference was never significant. These results showed that neither DR4 and DR7 alleles together nor DR7 alleles alone were associated with specific allergic reactivity to a specific allergen.

TABLE V. Patients reacting with Bet v 1, Bet v 2 (profilin), minor birch pollen allergens, Mal d 1 and Mal d 2 (apple allergens), and Dac g 1, Dac g 2, Dac g 3, Dac g 4, Dac g 5a, and Dac g 5b (*Dactylis* allergens) separated by isoelectric focusing and revealed by immunoprinting

Allergens	pI	Sensitive patients (n)	Sensitive patients (%)
Bet v 2	4.4-5.0	2	4.8
Bet v 1	5.0-5.6	41	97.6
a	5.9-6.1	12	28.6
b	6.2-6.5	7	16.6
c	6.7-6.9	17	40.5
d	7.0-7.2	6	14.3
e	9.7-10.0	6	14.3
Mal d 2	4.5-4.8	1	2.4
Mal d 1	5.0-6.0	34	81.0
Dac g 2	5.0-5.3	8	19.0
Dac g 5a	5.0-5.6	13	30.9
Dac g 1	6.1	24	57.1
Dac g 5b	6.9-7.8	14	33.3
Dac g 3	8.5-9.0	7	16.7
Dac g 4	9.5-10.0	8	19.0

Total number of patients: $n = 42$.
pI, Isoelectric point.

DISCUSSION

Previous case/control studies of the possible role of HLA class II antigens in atopy have been performed with particular pollen allergens and have yielded conflicting results.^{16,17} A limited occurrence of weak associations of HLA-DR alleles with IgE responses to few aeroallergens

TABLE VI. Distribution of HLA-DR4 and/or DR7 alleles or DR7 alleles only in patients with positive responses to birch pollen allergens compared with the distribution of the same alleles in patients allergic to additional allergens

Allergens	Patients with positive responses (n)	Patients with DR4 and/or DR7 alleles	P values	Patients with DR7 alleles	P values
Birch pollen	42	20	—	11	—
Birch pollen + apple	34	18	.30	10	.59
Birch pollen + grass	26	15	.18	9	.22

The number of patients with positive responses was evaluated by immunoblotting techniques, when clinical history, skin prick test responses, and IEF results were in good correlation (no significant difference between the number of sensitive patients).

was described in nuclear and extended families.¹⁸ However, in many association studies, because the frequencies of HLA class II antigens in control subjects were determined by using individuals randomly selected from the general population, the association of genetic factors with atopy may well have been obscured. Indeed, the prevalence of atopy is nearly 40%, and the frequency of DR4 and DR7 specificities in Western Europe is close to 30% in the general population.¹⁹ This makes the random sampling of individuals inadequate for association studies of genetic factors and atopy.

We based our results on a careful selection of appropriate control subjects. Although the control subjects were essentially included in our study through the use of a questionnaire, it is likely that this was not a cause of misclassification because all the volunteers subjected to skin prick tests had negative responses and the additional sera that could be tested by RASTs and immunoblotting showed negative results.

Our results show a strong association with atopy of the HLA class II-DR7 alleles in unrelated white patients, all of whom had a positive skin test reaction to at least Bet v 1. In white populations DR7 is known to be in strong linkage disequilibrium with allele DQA1*0201.¹⁹ However, the latter allele was no more significantly associated with the disease after *P* values were corrected for multiple comparisons. We could assume that DQA1*0201 is not independently associated with the disease and that only the DR7 allele is directly involved in the susceptibility to atopy. Although we failed to show any significant difference between the patient and control groups in the distribution of HLA-DR4 alleles when *P* values were corrected for multiple comparisons, we suggest that these alleles might represent a risk factor for the disease because the frequency of the affected subjects sharing both specificities or carrying either DR4 or DR7 alleles was very significantly higher than that found in the control group. This confirmed previous results with polysensitized allergic patients.³ Moreover, in a previous study² of a large and extensive family of 3 generations comprising 35 members, we reported that susceptibility to allergy, with or without asthma-related symptoms, was frequently associated with HLA class II DR4 and/or DR7 specificities.

No significant difference was observed by comparing the distribution of both risk alleles DR4 and/or DR7 in the 2 groups of allergic subjects with and without associ-

ated asthma symptoms. Although these results should be replicated in larger groups of subjects, we suggest that the latter alleles are more closely related to atopy than to asthma per se.

The question then arises as to whether the associations observed above with the latter alleles could be correlated with sensitivity to particular allergens. As seen from our results, all 42 patients were at least sensitive to the major allergen of birch pollen, Bet v 1; however, about 80% (*n* = 34) were also allergic to apples, and more than 60% were allergic to some grasses. When the clinical results and case histories of sufficiently large groups of allergic individuals are studied, and RAST and skin prick test results are compared with those of immunoblot analysis, evidence for reactivity against more than one allergen per subject is most often observed.

We have been unable to detect any significant positive association between the number of patients carrying either allele DR4 or DR7 and the sensitivity to an additional allergen other than Bet v 1. However, when we consider the group of patients with reactions to Bet v 1 and apples who were also allergic to olive tree pollen (results not shown), 7 of 8 subjects (about 90%) carried the risk alleles DR4 or DR7, and 6 carried DR7 exclusively. These results agree with those of Cardaba et al²⁰ who have shown that DR7 and DQ2 specificities are implicated in the recognition of Ole e 1, the major antigen from olive tree pollen. In this study the association with DR7 is statistically the strongest and is even significant when taking into account the multiple comparisons. We could therefore submit that HLA-DR7-encoded molecules are more noticeably involved in the presentation of food and pollen allergens than HLA-DR4.

Because no significant correlation was found between any specific antigen response and HLA class II DR4 and DR7 alleles, we suggest that the latter alleles are more associated with general atopic susceptibility. An explanation for this lack of stringent specificity is that a very similar structure in different or related proteins may act as an allergen in various and sometimes unrelated species, thereby reducing the apparently great diversity of allergens. Cross-reactivity between birch and apple sensitization could be explained by some previously obtained data²¹ showing that Bet v 1 shares common epitopes with a 17-kd protein of apple extracts. This is also consistent with the concept of a panallergen that might be responsible, in some patients, for the cross-allergic man-

ifestations caused by various or unrelated species of plant or food allergens. In recent years the highly conserved protein profilin (ie, Bet v 2) was identified as a cross-reactive allergen in pollen and vegetable foods.^{22,23} However, we could not confirm this hypothesis in the present work; only 2 of 42 patients reacted to profilin versus 20% in other reports.^{24,25} Likewise, in a previous study Valenta et al²⁶ observed only 3 patients reacting to Bet v 2 among a cohort of 100 subjects allergic to birch pollen.

Support for the mechanism of cross-sensitization has been further provided by other data. Some authors have recently shown that glycan epitopes could also be responsible for allergenic responsiveness against olive pollen in patients allergic to glycoproteins from other sources.²⁷ Therefore many explanations could exist for the observed cross-reactions between unrelated, nonhomologous allergens. This leaves the possibility that in the future other molecules would be identified as cross-sensitizing agents.

In summary, we confirm that HLA class II DR7 alleles are positively associated with a general susceptibility to atopy. Our results suggest that these alleles could be noticeably implicated not only in pollen and apple allergy but also in allergy to grass pollens.

Our study included complete HLA class II haplotype characterization in affected and unaffected subjects to discard the effects of linkage disequilibrium. It is important to point out that in previous studies we were able to replicate these associations in an extended family and with independent groups of unrelated individuals analyzed at different periods and recruited from different geographic areas.

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