

Evidence of a strong, positive association between atopy and the HLA class II alleles DR4 and DR7

Y. ARON, N. DESMAZES-DUFEU*, R. MATRAN*, B. S. POLLA, D. DUSSER*, A. LOCKHART and E. SWIERCZEWSKI

Laboratoire de Physiologie Respiratoire, Université Paris V and *Département of Pneumology, Hôpital Cochin, Paris, France

Summary

Background Atopy, with or without associated asthma, provides a useful model for evaluating the genetic factors that control human immune responsiveness. HLA class II gene products are involved in the control of immune responses.

Objectives We investigated whether susceptibility or resistance to the disease was associated with HLA class II genes.

Methods Blood samples were obtained from two groups of unrelated European-born white adults: 56 atopic patients (52 of them with asthma) and 39 healthy controls with no personal or familial history of asthma or atopy. Genomic DNA was extracted from peripheral blood lymphocytes. The exons of DQA1, DQB1, DRB and DPB1 genes were selectively amplified by the polymerase chain reaction (PCR) method. Genotyping was carried out by digestion of the amplified DNA products with allele-specific endonucleases (PCR-RFLP), which can recognize allelic variations in the polymorphic exon.

Results We found no significant differences in the frequency of DPB1 alleles between patients and controls. HLA class II DR4 and DR7 alleles were present in 39.2% of the patients and in 2.5% of the healthy subjects ($P_{c*2} \leq 3.9 \cdot 10^{-3}$). Conversely, DQA1*0103 and DQB1*0502 alleles were more frequent in the control subjects. These results confirm a previous study of an extended pedigree, which showed that DR4 and DR7 alleles were absent in all healthy members of the family and were frequently observed in atopic and/or in asthmatic subjects.

Conclusion We observed that HLA-DR 4 and DR7 alleles are significantly implicated in their susceptibility to the disease and suggest that this susceptibility is more related to atopy than to specific responses to allergens. According to previous studies, we could also submit that in atopic patients with asthma, DR4 alleles at the least, could be more closely associated with atopy than with asthma per se. Conversely, we suggest that some allelic DQA1 and DQB1 sequences might confer protection against the disease.

Keywords: atopy, asthma, case/control study, HLA class II genes

Clinical and Experimental Allergy, Vol. 26, pp. 821–828. Submitted 17 March 1995; revised 4 September 1995; accepted 16 January 1996.

Introduction

Numerous studies in the general population and within families have suggested that both genetic and environmental

influences are involved in the pathogenesis of asthma and atopy [1–3]. In twins the contribution of a genetic component was shown to be more substantial than that of familial environment [4]. Despite ample evidence for heritability, genetic analysis has been difficult and contradictory. In studies carried out on British populations, a major locus for atopy was localized on chromosome

Correspondence: Dr E. Swierczewski, Laboratoire de Physiologie Respiratoire, Faculté de Médecine Cochin Port Royal, 24 rue du Faubourg Saint-Jacques, 75014, Paris, France.

11q 13 [5,6], close to the gene coding for the beta subunit of the high-affinity immunoglobulin E (IgE) receptor. Furthermore, the same authors found a mutation point in this gene in 17% of the families with atopic probands [7]. However, other authors have been unable to confirm a significant link between this region and atopy as defined by increased total and specific IgE levels and positive skin-prick tests [8–11].

HLA class II genes coding for human leucocyte antigens class II molecules, are likely candidates for controlling the immune response to common allergens [12]. 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 [13,14]. Of all the results of these previous investigations only the association between HLA-DR and the minor ragweed allergen Amb V has been confirmed [15,16]. However, the success of association studies critically depends on the appropriate selection of controls. The lack of a strong association between allergic diseases and HLA class II genes observed in previous studies [17], could be due to the high prevalence (about 30%) of atopy in control subjects, who have been selected at random from the general population.

Recently, we have shown [18] that some HLA class II alleles may confer protection against isocyanate-induced asthma (IAA), and that IAA and extrinsic asthma probably differ in terms of their association with HLA class II genes. Moreover, in a study of a large and extensive French family of three 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 DR7 specificities [19].

The present study was designed to investigate whether these preliminary results could be reproduced in groups of unrelated individuals. To test this hypothesis, we studied the distribution of haplotypes and alleles in the different HLA class II genes in two groups of unrelated white European: allergic patients with or without asthma, and healthy asymptomatic control subjects.

In view of the importance of phenotype characterization in case/control studies, a careful selection of non-atopic control subjects was carried out.

Our results confirm that a strong association exists between some HLA class II alleles and the susceptibility to atopy. We also suggest that some HLA class II allelic sequences may contribute to protection against the disease.

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 whites living in the Paris area (France).

Fifty-six patients (35 females and 21 males; mean age 41.3 ± 2.26 year) were admitted in the study, which was conducted in the Department of Pneumology of Cochin Hospital. They were given a standardized questionnaire by physicians with extensive experience in allergic diseases.

All patients had a documented atopic allergy with or without asthma-related symptoms. Phenotype included medical history, typical asthma symptoms (shortness of breath, wheezing, coughing and chest tightness), and a bronchial hyperresponsiveness to methacholine challenge, administered by a breath-activated nebulizer (FDC, Model 88, Mediprom, France). The mean of forced expiratory volume/s (FEV₁) was $86\% \pm 3$ of the predicted values.

Subjects were defined as atopic if they were positive to one or more of the following criteria: positive skin-prick tests (as defined by a weal of ≥ 5 mm diameter) carried out with 17 common allergens consisting of *D. pteronyssinus* and *D. farinae*, common moulds, grass and tree pollens and animal danders; one negative control and two positive controls were also applied; a raised total IgE as defined by an IgE level of 2 sd above the predicted mean for the age of the subject; bronchial hyperresponsiveness was measured as described above.

We also studied 39 control subjects from the same geographical area (24 females and 15 males; mean age 42.7 ± 3.2 years). They 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 three generations. Skin tests ($n = 14$) and methacholine inhalation challenge ($n = 6$) were also performed on control volunteers.

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

Experimental protocol

DNA preparation

Blood samples (20 mL) were obtained from all individuals. Genomic DNA was extracted from the peripheral blood lymphocytes using the phenol-chloroform method [20]. In 50% of the cases, DNA was isolated from buccal epithelial cells by a micromethod already described [19].

Polymerase chain reaction (PCR)

The second exon of DQA1, DQB1, DPB1 and DRB genes was selectively amplified by the PCR method using a Hybaid thermal reactor (Teddington, UK). This reaction was performed in a volume of 100 μ L, containing 67 mM Tris-HCl, pH 8.8; 16.6 mM $\text{SO}_4(\text{NH}_4)_2$; 6.7 mM MgCl_2 ; 0.2 μ M of each primer; 10% v/v dimethylsulfoxide (DMSO), except for DQA1 typing; 0.25 mM of each dNTP; 1 μ g of template DNA and 2.5 units of Taq DNA polymerase (Cetus, Norwalk, CT, USA). Light mineral oil (Sigma) was added to the solution to avoid evaporation. After heating for 5 min at 94 °C and 2 min at 58 °C, the samples were subjected to 30 cycles of amplification by denaturation for 30 s at 94 °C, primer annealing for 50 s at 58 °C and extension for 30 s at 72 °C. PCR products were controlled in a 2% agarose gel stained with ethidium bromide.

PCR primers

The sequences of different primers used for DQA1, DQB1 and DPB1 genes amplification were described previously [21–24]. HLA DRB genes were amplified using the primers GH46 and GH50 [25] for the generic amplification. To discriminate between the different DRB1 alleles of the DR 3, 5, 6 and 8 haplotypes, the second exon of the DRB genes was amplified with the following primers: GAMPDRB1 and PL368. Selective amplification of DR4 alleles was achieved by the combined use of a group-specific primer PSP-25 and the DRB-specific primer GAMPDRB1, quoted above [26].

Analysis of the PCR products

Genotyping was carried out by digestion of the amplified DNA products with allele-specific endonucleases (PCR-RFLP), which can recognize allelic variations in the polymorphic exon. For each locus several allele-specific endonucleases were chosen.

The restriction endonucleases used were the following:

- DQA1 gene: Dde I, Rsa I and Fok I
- DQB1 gene: Acl I, Hpa II, Cfo I/and or Hin PI, Sau 961 and Hae III
- DPB1 gene: Rsa I, Ban II, Fok I, Eco NI, Dde I and Bst U I
- Generic DRB: Hinf I, Nsi I, Hph I and Dde I DRB (DR3, 5, 6, 8): Ava II, Asp 700, Hin PI, Rsa I and Apa I
- Specific DRB1 of DR4: Mn I, Hinf I, Hae II, Hph I, BstU I

Of PCR products, 8 μ L was digested separately, with four units of each enzyme under the conditions recommended by the manufacturers (Biolabs, Beverly, MA, USA; and Bøhringer, Mannheim, Germany), and the

products were size-analysed in a 10% polyacrylamide gel. The gel was stained in 1 μ g/mL of ethidium bromide and photographed under ultraviolet light.

HLA-typing

The PCR-RFLP method was used to determine the frequencies of alleles, haplotype and allelic combinations for four polymorphic loci in the different populations. Eight DQA1, 11 DQB1 and 20 DPB1 alleles could be identified. The use of three endonucleases clearly distinguishes six of eight DQA1 alleles, except for DQA*0101 and DQA1*0102, which are considered a unique group of alleles. The first step in the DRB-typing strategy allowed us to identify 10 DR specificities. During the second step, 12 DRB1 alleles associated with the DR3, DR5, DR6 and DR8 specificities could be discriminated. We also could determine eight subtypes of the DR4 family, using DR4 group-specific amplification.

Statistical analysis

Frequencies of each allele were compared using the chi-square test. Relative risk (RR) was calculated using the Wolf test with Haldane's modification [27,28]. For *P*-value determination, Fisher's exact test was utilized and the level of significance was set to 0.05. *P*-value was corrected for the number of alleles tested.

Results

Clinical and functional data

All 56 patients with atopic allergy had a documented history of symptoms related to at least one atopic disease: 52 had asthma and the remaining four had allergic rhinitis and/or atopic eczema. Among the 24 asthmatic subjects who underwent a bronchial challenge to methacholine, a 20% fall in FEV_1 was observed in 21 of them based on a group average of methacholine ($\text{PD}_{20} \text{FEV}_1$) of $580 \pm 170 \mu\text{g}$. In the other patients, FEV_1 was too low to permit the methacholine challenge to be performed safely. Fifty per cent of the patients demonstrated skin reactivity to at least three or more allergens, and 41% to one or two allergens. Only three patients had negative prick tests. Total serum IgE was measured in 38 patients, of which only 34% demonstrated a raised total IgE ($\geq 300 \text{ UI/L}$).

For ethical reasons skin-prick tests and respiratory challenge tests were only performed on healthy volunteers. Among the 39 control subjects, skin-prick tests were carried out on 14 individuals. All were negative. All control volunteers ($n = 6$) who were submitted to non-

Table 1. Distribution of HLA-DRB1 alleles among patient and control group

Alleles	Patient group (n = 56)		Control group (n = 39)		P	Pc*
	n	%	n	%		
DR1	6	10.7	5	12.8	NS	NS
DR2	16	28.5	18	46.1	NS	NS
DR3	5	8.9	8	20.5	≤ 0.03	NS
DR4	22	39.2	1	2.5	$\leq 4.10^{-5}$	$\leq 4.10^{-4}$
DR5	21	37.5	13	33.0	NS	NS
DR6	19	33.9	16	41.0	NS	NS
DR7	12	21.4	0	0	$\leq 5.10^{-3}$	$\leq 5.10^{-2}$
DR8	6	10.7	1	2.5	NS	NS
DR9	2	3.6	0	0	NS	NS
DR10	1	1.8	5	12.8	NS	NS

NS. Not significant. Pc* = P corrected for the number of alleles tested.

specific inhalation challenge tests did not respond to the highest dose of methacholine used (3100 μ g).

Distribution of DR alleles

The distribution of DR alleles among the patients and the control subjects is shown in Table 1. The frequency of subjects carrying HLA-DR4 alleles was significantly higher ($Pc^{*2} \leq 4.10^{-4}$) among the patient group (39.2%) than among the controls (2.5%). When the frequency of the affected subjects carrying either DR4 or DR7 alleles, or sharing both specificities (34 of 56 patients), was compared with the healthy controls (1 of

39 control subjects), the difference was quite significant ($Pc^{*2} \leq 3.9 \cdot 10^{-3}$). No significant differences were noted between frequencies of DR4 subsets in the patient group, alleles DRB1*0401,*0402,*0403,*0404 and*0408 being prevalent.

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

Distribution of DQA1 alleles

A significant increase in allele DQA1*0301 was apparent in the patients ($Pc^{*2} \leq 2.10^{-3}$) compared with the

Table 2. Distribution of DQA1 alleles among patient and control group

DQA1	Patient group (n = 55)		Control group (n = 37)		P	Pc*
	n	%	n	%		
*0101						
*0102	27	49	23	62	NS	NS
0103	7	12.7	11	20.7	≤ 0.043	NS
0201	13	23.6	2	5.4	≤ 0.026	NS
0301	24	43.6	3	8.1	$\leq 2.10^{-4}$	$\leq 2.10^{-3}$
0401	3	5.4	3	8.1	NS	NS
0501	25	45.4	24	64.8	NS	NS
0601	0	0	0	0	NS	NS

NS. Not significant. Alleles DQA1*0101 and *0102 could not be differentiated; Pc* = P corrected for the number of alleles tested.

Table 3. Distribution of DQB1 alleles among patient and control group

DQB1	Patient group (<i>n</i> = 55)		Control group (<i>n</i> = 36)		<i>P</i>	<i>P_c</i> *
	<i>n</i>	%	<i>n</i>	%		
0201	14	25.4	9	25	NS	NS
0301	17	30.9	10	27.7	NS	NS
0302	21	38.2	3	8.3	$\leq 1.10^{-3}$	$\leq 1.10^{-2}$
0303	6	10.9	0	0	4.10^{-2}	NS
0401°						
0402°	2	3.6	0	0	NS	NS
0501	8	14.5	9	25	NS	NS
0502	2	3.6	6	16.6	NS	NS
0503	0	0	3	8.3	NS	NS
0601	1	1.8	0	0	NS	NS
0602°	20	36.3	13	36	NS	NS
0603°						
1604	5	9	6	16.6	NS	NS
0605	6	10.9	2	5.5	NS	NS

NS. Not significant. °Alleles DQB1*0401 and *0402 and DQB1*0602, *0603 could not be differentiated; *P_c** = *P* corrected for the number of alleles tested.

control subjects (Table 2). By contrast, the frequency of allele DQA1*0103 was increased in the controls; however, this difference did not reach statistical significance when *P* was corrected for multiple comparisons.

Distribution of DQB1 alleles

As seen in Table 3, the frequency of the allele DQB1*0302 was significantly increased ($P_{c*}^2 \leq 1.5 \cdot 10^{-2}$) in the atopic individuals compared with the control

subjects. Conversely, allele DQB1*0502 showed a tendency to increase in the control group; however, the difference was no more significant when the *P*-value was 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).

Table 4. Distribution of HLA class II alleles positively and negatively associated with atopy in the general French population¹ and in our non-atopic control subjects

	Selected controls		Random French population ¹		<i>P_c</i> *
	<i>n</i>	%	<i>n</i>	%	
A°DR4 + DR7	1 (39)	2.5	42 (158)	27.0	$\leq 1.10^{-2}$
DQA1*0301	3 (37)	8.1	26 (181)	14.4	NS
DQB1*0302	3 (36)	8.3	14 (181)	7.7	NS
B°DQA1*0103	11 (37)	29.7	11 (181)	6.0	$\leq 5.0 \cdot 10^{-4}$
DQB1*0502	6 (36)	16.6	5 (181)	3.0	$\leq 3.0 \cdot 10^{-2}$

¹Data from J. Colombani, [29] (with author's permission).

A°. Susceptibility alleles. B°. Protective alleles. Figures in brackets represent the total number of subjects in each group; *P_c** = *P* corrected for the number of alleles tested.

In Table 4, we compare the distribution of those HLA class II alleles, that are positively or negatively associated with allergic disorders, in two different groups of subjects: in a randomly recruited French population which has been described in previous studies [29] and in our group of selected non atopic control subjects. The frequencies of both HLA class II specificities, associated with DR4 and DR7-risk alleles, were much higher (27%) in the random French population than in our selected control group (2.5%). However, no significant difference was observed between both groups, in the distribution of the allele DQA1*0301. Conversely, frequencies of the alleles DQA1*0103 and DQB1*0502, which are negatively associated with disease, were significantly higher ($P_{c^2} \leq 3.5 \cdot 10^{-4}$ and $4 \cdot 10^{-2}$, respectively), in our selected control group compared with the randomly recruited subjects.

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 [30, 31]. Young *et al.* [32] described in nuclear and extended families, a limited occurrence of weak associations of HLA-DR alleles with IgE response to six aeroallergens. However, in this family study, as in many association studies so far, since the frequencies of HLA class II antigens in controls were determined using individuals which had been 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 [5]. 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 controls. Although the control subjects were essentially included in our study through use of a questionnaire, it is likely that this was not a cause of misclassification, for the reason that all volunteers subjected to respiratory challenge and/or skin-prick tests, elicited negative responses.

Our results show a strong association with atopy, in unrelated white patients, of the HLA class II alleles DR4 and/or DR7 and of the alleles DQA1*0301 and DQB1*0302. These observations suggest that either the whole haplotype DR4/DQA1*0301/DQB1*0302 or only some alleles of this haplotype are risk factors for the disease. In white populations, DR4 is known to be in strong linkage disequilibrium with alleles DQA1*0301 and DQB1*0302 [29]. Moreover, as we have shown

above, the frequencies of the alleles DR4 and DR7 were significantly lower in our group of non-atopic control individuals than in a randomly selected population, whereas the distribution of DQA1*0301 and DQB1*0302 alleles remained almost similar in both groups. Therefore, we could assume that there is no independent association of DQA1*0301 and DQB1*0302 alleles with the disease and that only DR4 and/or DR7 alleles are directly involved in the susceptibility to atopy.

Although we failed to show a significant difference in the distribution of DQA1*103 and DQB1*502 alleles between patient and control groups, when *P* was corrected for multiple comparisons, the frequency of these alleles increased significantly in the selected controls compared with the random population. This suggests that these alleles could play a role in protection against the disease.

The question then arises whether some of these associations were related to atopy or to asthma per se. Although most of our patients suffered from both asthma and allergy, we could assume that the DR4 alleles in particular, are more likely to be associated with immune response to allergens. This is consistent with our previous findings of a very low frequency of HLA-DR4 alleles in isocyanate-sensitive asthmatic patients, which were non-atopic in most cases [18].

Since the majority of patients involved in our case/control study were polysensitized, the nature of specific responses to given aeroallergens could not be determined. However, we suggest that DR4 and DR7 alleles are more involved in a general susceptibility to atopy than in a specific sensitivity to particular allergens.

This hypothesis is supported by the results of an ongoing study [33] with patients originating from eastern France, which are allergic to birch pollen and specific foods. In this work, we found the same positive and negative associations with HLA class II alleles, that we found in the present study. Furthermore, these data were reproduced in a preliminary study concerning a small group of patients diagnosed with allergic bronchopulmonary aspergillosis (unpublished results).

Foreign aeroallergens are proteins with a complex structure which are endocytosed and degraded to small peptides, before binding to the groove of the MHC molecules. Recent observations of MHC class I molecules are consistent with the fact that only some residues of the peptides could extend into the binding groove of HLA molecules. This leaves many possibilities for variability in the remaining residues [34] and could explain why sensitization to many different molecules could be associated with only few restricted haplotypes and/or alleles, although aeroallergens differ widely in structure.

A second 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. This is consistent with the concept of a panallergen which is responsible in some patients for cross-allergic manifestations caused by various or unrelated species of plant or food allergens [35,36].

In summary, we confirm that HLA class II DR4 and DR7 alleles are positively associated with atopy. We also suggest that some DQB and DQA alleles might be protective against the disease. Our study included complete HLA class II haplotype characterization in affected and unaffected subjects, in order to discard the effects of linkage disequilibrium. Finally it is important to point out that in ongoing studies, we were able to replicate these associations with independent groups of individuals, analysed at different periods and recruited in different geographical areas.

Atopy and asthma are multifactorial diseases arising from complex interactions between genetic and environmental factors. Other susceptibility or resistance genes located either close to or outside of the HLA region could also play a role, e.g. genes involved in regulating the assembly of the MHC/peptide complex; the antigen-processing pathway [37], or the IgE responsiveness [7].

Acknowledgements

This work was supported by Grant N° 92 FGA/3 from the Comité National Contre les Maladies Respiratoires, and by a grant from the Association Claude Bernard. We are grateful to Dr I. Fajac for her help in establishing the clinical phenotypes of patients. We thank Mrs S. Lemesre for her skillful technical assistance.

References

- Cooke RA, van der Veer A. Human sensitization. *J Immunol* 1916; 1:201–305.
- Gerrard JW, Vickers P, Gerrard CD. The familial incidence of allergic disease. *Ann Allergy* 1976; 36:10–15.
- Edfors-Lubs M.L. Allergy in 7000 twins pairs. *Acta Allergologica* 1971; 26:249–85.
- Hanson B, Mc Gue M, Roitman-Johnson B et al. Atopic disease and immunoglobulin E in twins reared apart and together. *Am J Hum Genet* 1991; 48:873–9.
- Cookson W, Hopkin J. Dominant inheritance of atopic immunoglobulin E responsiveness. *Lancet* 1988; i:86–8.
- Cookson W, Sharp P, Faux J, Hopkin J. Linkage between immunoglobulin E responses underlying asthma and rhinitis and chromosome 11q. *Lancet* 1989; i:1292–5.
- Shirakawa T, Airong L, Dubowitz M et al. Association between atopy and variants of the B subunit of the high affinity immunoglobulin E receptor. *Nature Genetics* 1994; 7:125–30.
- Hizawa N, Yamaguchi E, Ohe M et al. Lack of linkage between atopy and locus 11q13. *Clin Exp Allergy* 1992; 22:1065–9.
- Rich SS, Roitman-Johnson B, Greenberg B, Roberts S, Blumenthal MN. Genetic analysis of atopy in three large kindreds: no evidence of linkage to D11S97. *Clin Exp Allergy* 1992; 22:1070–6.
- Amelung PJ, Panhuysen CIM, Postma DS et al. Atopy, asthma and bronchial responsiveness: exclusion of linkage markers on chromosome 11q and 6p. *Clin Exp Allergy* 1992; 22:1077–84.
- Lympny PI, Welsh KI, Cochrane GM, Kemeny DM, Lee TH. Genetic analysis of the linkage between chromosome 11q and atopy. *Clin Exp Allergy* 1992; 22:1085–92.
- Charron D. Molecular basis of human leucocytes antigen class II disease associations. *Adv Immunol* 1990; 48:105–59.
- Ansari AA, Freidhoff LR, Myers DA, Bias WB, Marsh DG. Human immune responsiveness to *Lolium perenne* pollen allergen Lol pIII (Rye III) is associated with HLA-DR3 and DR5. *Hum Immunol* 1989; 25:59–71.
- O'Hehir RE, Eckels DD, Frew AJ, Kay AB, Lamb JR. MHC-class II restriction specificity of cloned human T lymphocytes reactive with *Dermatophagoides farinae* (house dust mite). *Immunology* 1988; 64:627–31.
- Marsh DG, Hsu SH, Roebber M et al. HLA-Dw2: a genetic marker for human immune response to short ragweed pollen allergen Ra5. 1. Response resulting primarily from antigenic exposure. *J Exp Med* 1982; 155:1439–51.
- Roebber M, Klapper DG, Goodfriend et al. Immunological and genetic studies of Amb i V (Ra5 G) an Ra homologue from giant ragweed pollen. *J Immunol* 1985; 134:3062–9.
- Howell WM. HLA immunogenetics and specific IgE responses to allergens. *Clin Exp Allergy* 1994; 24:401–4.
- Bignon JS, Aron Y, Ju LY et al. HLA class II alleles in isocyanate-induced asthma. *Am J Respir Crit Care Med* 1994; 149:71–5.
- Aron Y, Swierczewski E, Lockhart A. A simple and rapid method for genomic DNA extraction from jugal epithelial cells. Application to human lymphocyte antigen typing in one large family of atopic/asthmatic probands. *Allergy* 1994; 49:788–90.
- Sambrook J, Fritsch EF, Maniatis T. *Molecular Cloning*. 2d ed. Cold Spring Harbor Laboratory Press, 1989; 9.14–9.23.
- Scharf SJ, Horn GT, Erlich HA. Direct cloning and sequence analysis of enzymatically amplified sequences. *Science* 1986; 233:1076–8.
- Todd JA, Bell JI, Mc Devitt HO. HLA-DQB gene contributes to susceptibility and resistance to insulin-dependent diabetes mellitus. *Nature* 1987; 329:599–600.
- Bugawan TL, Horn GT, Long CM et al. Analysis of HLA DP allelic sequence polymorphism using in vitro enzymatic DNA amplification of DP-alpha and DP-beta loci. *J Immunol* 1988; 141:4024–30.

- 24 Ju LY, Gu XF, Layer E, Krishnamoorthy R, Charron D. Application of silver staining to the rapid typing of the polymorphism of HLA-DQ alleles by enzymatic amplification and allele-specific restriction fragment length polymorphism. *Electrophoresis* 1991; 12:270-3.
- 25 Scharf SJ, Long EO, Erlich HA. Sequence analysis of the HLA-DRB and HLA-DQB loci from three Pemphigus vulgaris patients. *Hum Immunol* 1988; 21:61-9.
- 26 Ju LY, Gu XF, Bardie R, Krishnamoorthy R, Charron D. A simple, non radioactive method of DNA typing for subsets of HLA- DR:prevalence data on HLA-DR4 subsets in three diabetic population groups. *Human Immunol* 1991; 31:251-8.
- 27 Haldane JBS. The estimation and significance of the logarithm of a ratio of frequencies. *Ann Hum Genet* 1956; 20:309-11.
- 28 Busson M, Prevost P. Contribution of statistics to the study of association and linkage between HLA and diseases. *Path Biol* 1986; 34:711-4.
- 29 Colombani J. HLA. Fonctions immunitaires et applications médicales. John Libbey Eurotext, 1993.
- 30 Levine BB, Stember RH, Fontino M. Ragweed hayfever: genetic control and linkage to HLA haplotypes. *Science* 1972; 178:1201-3.
- 31 Fischer GF, Pickl WF, Fae I et al. Association between IgE response against Bet V I, the major allergen of birch pollen, and HLA DRB alleles. *Hum Immunol* 1992; 3:259-65.
- 32 Young RP, Dekker JW, Wordsworth BO et al. HLA-DR and HLA-DP genotypes and immunoglobulin E responses to common major allergens. *Clin Exp Allergy* 1994; 24:431-9.
- 33 Aron Y, Geny S, Bessot JP et al. HLA class II-DRB haplotypes associated with asthma and allergy to birch pollen and food. 8th. European Histocompatibility Conference, March 7-9, Strasbourg, 1994, 203.
- 34 Corrigan CJ. Genetics of regulation of the human immune response: Report on a symposium held at Ringberg Castle. *Clin Exp Allergy* 1992; 22:869-75.
- 35 Valenta R, Duchene M, Pettenburger K et al. Identification of profilin as a novel pollen allergen; IgE-autoreactivity in sensitized individuals. *Science* 1991; 253:557-60.
- 36 Valenta R, Duchene M, Ebner C et al. Profilins constitute a novel family of functional plant pan-allergens. *J Exp Med* 1992; 175:377-85.
- 37 Kuwata S, Yanagisawa M, Saeki H et al. Polymorphisms of transporter associated with antigen processing genes in atopic dermatitis. *J Allergy Clin Immunol*. 1994; 94:565-74. Suppl.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.