

# Lack of evidence of a significant association between HLA-DR, DQ and DP genotypes and atopy in families with HDM allergy

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## Summary

**Background** HLA class II genetic polymorphism has been variably implicated in susceptibility to specific immune responsiveness to house dust mite (HDM) allergens, and may also influence the development of atopy.

**Objective** In order to assess accurately the influence of HLA alleles in the atopic immune response, we typed 22 families selected from 131 previously obtained randomly selected families (i.e. without regard to atopy or asthma), chosen on the basis of two or more members having skin prick reactivity to HDM.

**Methods** Each individual was fully typed for HLA-DRB1, DQA1, DQB1 and DPB1 class II alleles using a combination of sequence-specific oligonucleotide probes (SSOP) and sequence-specific primer (SSP) polymerase chain reaction (PCR) typing and direct sequencing.

**Results** Using appropriate statistical tests, no significant allelic associations were found between any DRB1, DQB1 or DPB1 alleles and atopy or skin prick reactivity to *Dermataphagoides pteronyssinus* (Der p) or *D. farinae* (Der f). However, positive associations were found between DQA1\*0301 and skin prick reactivity to Der f ( $P=0.009$ ) and atopy ( $P=0.027$ ). Sib-pair analysis revealed no significant sharing of alleles between affected sib pairs for any of the phenotypes studied.

**Conclusion** These results fail to confirm a previously reported association between the DRB1\*04 and 07 haplotypes and atopy, and suggest that HLA class II restriction does not play a major role in the development of the IgE response to domestic house dust mite allergens in the British population.

**Keywords:** HLA, atopy, HDM allergy

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## Introduction

It is well established that IgE directed to common environmental aero-allergens including those derived from domestic house dust mites (HDM), plays a major role in the pathogenesis of asthma and atopic allergic

disorders. The phenotype of atopy is multivariate, including total and specific IgE titres and skin prick tests to allergens, and determination of atopic disorders through clinical history and examination and provocation tests of bronchial reactivity. Several genetic loci have been identified that are possibly involved in the regulation of the atopic immune response. Initial studies have suggested that the  $\beta$ -chain of the high-affinity IgE receptor on chromosome 11q13 is linked to atopy, with a strong bias towards maternal inheritance [1–5]. However, this

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finding has not been confirmed in subsequent studies [6–9]. More recently, the interleukin-13 (IL-13)/4/5/granulocyte–macrophage colony-stimulating factor (GM-CSF) cluster on chromosome 5q31 [10,11] has been shown to be linked to high total IgE levels and bronchial hyper-responsiveness [12]. While these and possibly other genes appear to control the level of the atopic immune response, there are a number of other genetic influences that are more allergen specific and may also be important in the development of the allergic response. Human leucocyte antigen (HLA) class II genes of the major histocompatibility complex (MHC), encoded on chromosome 6p, have been implicated in the susceptibility to a number of diseases known to have an immunological basis [13], and recently a number of studies have found HLA associations with specific IgE responses to several allergens.

The HLA class II region is composed of DR, DQ, DP subregions, each of which consists of at least one A and one B gene that encode the  $\alpha$ - and  $\beta$ -chains of the expressed cell-surface molecules. Both the A and B genes may be polymorphic, but most polymorphism resides in the B genes. Currently there are two DRA, 126 DRB, 12 DQA, 22 DQB, six DPA and 56 DPB alleles recognized that are differently expressed [14]. The expressed class II molecules (largely found in cells involved in the immune response) function in the presentation of intracellularly processed peptides to CD4<sup>+</sup> T-helper cells.

Initial studies of HLA class II associations with specific IgE responses concentrated on small well-defined antigens with a few epitopes, such as the ragweed allergen Amb a V and the rye grass allergen Lol p III [15–19], and showed that elevated IgE production to Amb a V occurs in individuals expressing DRB1\*1501 (DR2)-associated haplotypes. Further studies have established that HLA-DRB1, DRB3 and DRB5 genes are able to restrict the recognition of house dust mite (*Dermaphagoides* spp.) determinants [20], in line with population associations demonstrated at the Eleventh International Histocompatibility Workshop [21]. Other notable studies have suggested associations between DRB1\*0701, DQB1\*0201 and IgE responsiveness to the main olive pollen allergen Ole e I [22], DRB3\*0101 and IgE responsiveness to the birch pollen allergen Bet v I [23], and DQB1\*01 and the IgE responsiveness to the cat allergen Fel d I [24]. A number of recent studies have also demonstrated HLA class II associations in industrial asthma, where the triggering molecules are simple, low molecular weight molecules such as isocyanates and acid anhydrides [25,26].

However, few genetic epidemiological studies in atopic subjects have demonstrated significant associations between HLA class II genes and atopy and allergic

asthma. Blumenthal *et al.* [27], in a study of 52 patients with ragweed pollen allergy, found that DR2 (HLA-B7, DR2 extended haplotype) was associated with allergic asthma but not rhinitis, and that the HLA-B8, DR3 haplotype was associated with rhinitis only. More recently Aron *et al.* [28] found that in a large extended French white family of 33 individuals, DRB1\*04 and DRB1\*07 (DR53-associated haplotypes) were associated with atopy and asthma, a finding substantiated in a further case-control study of French white individuals [29].

In this study, in order to examine more fully the previously reported association between atopy and DRB1\*04 and 07, a series of 22 families showing HDM allergy in another well-defined white population (British) was investigated. A family-based study was used to allow sib-pair analysis to be performed, in addition to a search for HLA class II population associations. Comprehensive DNA-based class II typing for the DRB1, DQA1, DQB1 and DPB1 loci was performed, allowing a more precise assignment of HLA class II genotypes than in previous population studies [e.g. 27], which have largely relied upon serological typing methods.

## Materials and methods

### Subjects

Twenty-two families were selected from 131 previously obtained randomly selected families (i.e. without regard to atopy or asthma) with three or more children, on the basis of two or more members having skin prick test reactivity to HDM [*D. pteronyssinus* (Der p) or *D. farinae* (Der f); allergens were supplied by Bayer Corporation, Spokane, WA, USA]. A positive skin prick test was defined as  $\geq 3$  mm diameter of control. Atopy was defined as the log serum IgE level, the principal component of the following indices [30]: log serum total IgE, as measured by a standard radioallergosorbent test (RAST); serum-specific IgE, as measured by skin prick test to 12 common allergens and controls; the bronchial response to inhaled histamine acid phosphate, defined as the provocative concentration of inhaled agonist that reduced FEV<sub>1</sub> by 20% of baseline; and a clinical history of eczema, hayfever or wheeze, as determined by answers to a detailed questionnaire based on the IUATLD standard for adults and the ISAAC protocol for children [31,32]. Log serum total IgE had a correlation with atopy of 0.98 [30].

### HLA typing

Genomic DNA was extracted from peripheral blood

leucocytes by a rapid salt precipitation method [33]. HLA-DRB1, DQA1, DQB1 and DPB1 typing was achieved using polymerase chain reaction (PCR) amplification of the second (variable) exon of the class II gene. Primers and amplification conditions were as previously described [34,35].

Following dot-blot preparation of amplified DNA, sequence-specific oligonucleotide probe (SSOP) typing was performed using panels of oligonucleotide probes selected from the Eleventh International Histocompatibility Workshop panel [35]. These probes were DRB1-17 probes (1001, 1004, 1006-9, 2803, 2809, 3703, 3709, 5703-4, 7001, 7004, 7007, 8602 and DR8), DQA1-10 probes (2502, 3401-3, 4102, 5501-4, 6903), DQB1-17 probes (2301-2, 2601, 2603-4, 2606, 3702, 4501, 4901, 5701-7, 0504) and DPB1-19 probes (0901-4, 3503, 5501-4, 6901-6, 7601-2, 8501, 8503). All probes were 3' end-labelled with digoxigenin (DIG-11-ddUTP). Dot-blot membranes were blocked and prehybridization was performed using the method of Nevinny-Stickel *et al.* [36]. The chemiluminescent substrate CSPD (disodium 3-(4-methoxy-spiro {1,2-dioxetane-3,2''-5'-chloro} tricyclo [3.3.1.1<sup>3,7</sup>] decan} -4-yl) phenyl phosphate) (Boehringer Mannheim, Mannheim, Germany) was used to detect bound probe, visualized by exposure of X-ray film for 15 min.

HLA typing by allele-specific PCR-sequence-specific primer (SSP) methods [37-39] was used to genotype samples for which a complete type for DRB1 or DQB1 was unobtainable using SSOP. Briefly, this involves using 32 PCR reactions for each individual, 20 for assigning DRB1 genotype, three for DRB3, four and five (DR51-53 superspecificities) and eight for DQB1 together with a negative control. Each reaction mix contained two to four allele-specific primers and a positive internal control primer pair: for DRB1 the primers amplified the third intron of DRB1 genes and for DQB1 a primer pair that amplified a human growth hormone sequence was used.

Direct sequencing was used to obtain the genotype of samples for which a DPB1 type was not available through conventional SSOP typing [40]. Genomic DNA samples were amplified using primers that amplified the second exon of the DPB1 gene, one primer of the pair being biotinylated. PCR products were captured and purified using Dynabead M-280 magnetic streptavidin beads (Dynal, Oslo, Norway) and rendered single stranded by alkaline denaturation. Sequenase dye primer chemistry was used for sequencing using two internal fluorescently labelled primers (forward and reverse). Sequence ladder chromatograms were generated using an Applied Biosystems 373 automated sequencer (Applied Biosystems, Foster City, CA, USA).

The chromatograms were analysed using a Factura and Sequence Navigator computer software package (Applied Biosystems).

#### Statistical analyses

HLA-DRB1, DQA1, DQB1 and DPB1 phenotype frequencies were scored and  $2 \times 2$  contingency tables were constructed for all alleles between affected and non-affected individuals for all three phenotypes (skin prick reactivity to Der p, Der f and atopy defined as the log serum IgE level, the principal component of a regression analysis [30]) using the Epi Info v. 6.01 computer program (CDC, Atlanta, USA). Significance was evaluated using the  $\chi^2$  test or the Fisher's exact test if any expected value in a  $2 \times 2$  table was less than 5. Probability values for linkage were corrected for the number of comparisons made and the level of significance was set at  $P = 0.05$ . Haplotype sharing among affected sib pairs was analysed for skin prick test reactivity to Der p, Der f and atopy. Haplotype sharing was only counted if all four parental haplotypes were unambiguously identified, and sib pairs from multiple sibships were treated as independent. For non-linkage the expected sharing of haplotypes was expected to be 2:25% 1:50% 0:25%. An excess sharing from that expected was tested for by  $\chi^2$  estimation.

#### Results

The distribution of HLA class II single locus genotypes in Der p and Der f skin prick test-positive and atopic individuals is shown in Tables 1-4. There were no significant differences in allelic frequency between the subject population and unrelated 'healthy' control populations previously typed in our laboratory, consisting of unrelated British white cadaveric renal and bone marrow donors [41,42] (e.g. DRB1\*0401  $\chi^2 = 7.43$ ,  $P = 0.083$  when corrected for the number of alleles at the locus, DRB1\*0301  $\chi^2 = 0.27$ ,  $P = \text{ns}$ ). In addition no significant differences were seen between the allelic frequencies in non-atopics and those found in the unscreened control population. No significant associations were detected between any DRB1, DQB1 or DPB1 alleles and skin prick reactivity to either Der p, Der f or log serum total IgE. The strongest associations were seen between the serum IgE level and DQA1\*0301 ( $\chi^2 = 7.70$ ,  $P = 0.027$  when corrected for the number of alleles at the locus), and Der f skin prick test reactivity and DQA1\*0301 ( $\chi^2 = 9.47$ ,  $P = 0.009$ ) and DQB1\*0303 ( $\chi^2 = 7.46$ ,  $P = 0.088$ , ns). However, sib-pair analysis failed to reveal any increase in allele sharing in affected sib pairs over non-affected pairs for all three phenotypes studied.

**Table 1.** HLA-DRB1 genotype frequencies and skin prick reactivity and atopy in affected and non-affected individuals (*n* (%))

| DRB1                | Der p     |         | Der f     |           | Atopy     |           |
|---------------------|-----------|---------|-----------|-----------|-----------|-----------|
|                     | +         | -       | +         | -         | +         | -         |
| 01                  | 6 (5)     | 5 (5)   | 7 (5.1)   | 4 (4.9)   | 7 (5.4)   | 4 (4.4)   |
| 15                  | 24 (20)   | 11 (11) | 29 (21)   | 6 (7.3)   | 23 (17.7) | 12 (13.3) |
| 16                  | 1 (0.8)   | 1 (1)   | 1 (0.7)   | 1 (1.2)   | 2 (1.5)   | 0 (0)     |
| 03                  | 24 (20)   | 21 (21) | 30 (21.7) | 15 (18.3) | 28 (21.5) | 17 (18.9) |
| 04                  | 14 (11.7) | 22 (22) | 16 (11.6) | 20 (24.4) | 18 (13.8) | 18 (20)   |
| 11                  | 8 (6.7)   | 2 (2)   | 8 (5.8)   | 2 (2.4)   | 7 (5.4)   | 3 (3.3)   |
| 12                  | 6 (5)     | 3 (3)   | 7 (5.1)   | 2 (2.4)   | 6 (4.6)   | 3 (3.3)   |
| 13                  | 10 (8.3)  | 9 (9)   | 8 (5.8)   | 11 (13.4) | 11 (8.5)  | 8 (8.9)   |
| 14                  | 2 (1.7)   | 2 (2)   | 3 (2.2)   | 1 (1.2)   | 2 (1.5)   | 2 (2.2)   |
| 07                  | 14 (11.7) | 9 (9)   | 16 (11.6) | 7 (8.5)   | 15 (11.5) | 8 (8.9)   |
| 08                  | 4 (3.3)   | 3 (3)   | 5 (3.6)   | 2 (2.4)   | 4 (3.1)   | 3 (3.3)   |
| 09                  | 4 (3.3)   | 7 (7)   | 3 (2.2)   | 8 (9.8)   | 3 (2.3)   | 8 (8.9)   |
| 10                  | 3 (2.5)   | 5 (5)   | 5 (3.6)   | 3 (3.7)   | 4 (3.1)   | 4 (4.4)   |
| Totals ( <i>n</i> ) | 120       | 100     | 138       | 82        | 130       | 90        |

(Table 5) (serum IgE  $\chi^2 = 3.18$  (NS), Der p  $\chi^2 = 5.34$  (NS), Der f  $\chi^2 = 1.39$  (NS).

### Discussion

In this study a series of families showing HDM allergy in a well-defined British white population was examined. The use of a family-based study allowed sib-pair analysis to be performed in addition to a search for HLA class II population associations. Most previous studies examining

HLA associations with atopy or specific IgE responses have been based on unrelated clinically atopic individuals, with the exception of the studies by Young *et al.* [24] and Aron *et al.* [28]. In population studies up to 40% of unrelated 'healthy' controls may be atopic [43], which may mask any actual association with atopy. Family-based studies where all individuals have been screened for the atopic phenotype enable a true comparison of atopics versus non-atopics. In this study we used comprehensive DNA-based typing for the four most polymorphic HLA

**Table 2.** HLA-DQA1 genotype frequencies and skin prick reactivity and atopy in affected and non-affected individuals (*n* (%))

| DQA1*               | Der p     |         | Der f     |            | Atopy     |                        |
|---------------------|-----------|---------|-----------|------------|-----------|------------------------|
|                     | +         | -       | +         | -          | +         | -                      |
| 0101                | 10 (8.3)  | 12 (12) | 14 (10.1) | 8 (9.8)    | 12 (9.2)  | 10 (11.1)              |
| 0102                | 28 (23.3) | 16 (16) | 31 (22.5) | 13 (15.9)  | 28 (21.5) | 16 (17.8)              |
| 0103                | 4 (3.3)   | 3 (3)   | 4 (2.9)   | 3 (3.7)    | 6 (4.6)   | 1 (1.1)                |
| 0201                | 13 (10.8) | 8 (8)   | 15 (10.9) | 6 (7.3)    | 14 (10.8) | 7 (7.8)                |
| 0301                | 20 (16.7) | 28 (28) | 21 (15.2) | 27 (32.9)* | 20 (15.4) | 28 (31.1) <sup>†</sup> |
| 0401                | 2 (1.7)   | 1 (1)   | 2 (1.4)   | 1 (1.2)    | 1 (0.7)   | 2 (2.2)                |
| 0501                | 37 (30.8) | 24 (24) | 44 (31.9) | 17 (20.7)  | 42 (32.3) | 19 (21.1)              |
| 0601                | 2 (1.7)   | 2 (2)   | 3 (2.2)   | 1 (1.2)    | 3 (2.3)   | 1 (1.1)                |
| Untyped             | 4 (3.3)   | 6 (6)   | 4 (2.9)   | 6 (7.3)    | 4 (3.1)   | 6 (6.7)                |
| Totals ( <i>n</i> ) | 120       | 100     | 138       | 82         | 130       | 90                     |

\* $\chi^2 = 9.47$ ,  $P = 0.009$ .

<sup>†</sup> $\chi^2 = 7.70$ ,  $P = 0.027$ .

**Table 3.** HLA-DQB1 genotype frequencies and skin prick reactivity and atopy in affected and non-affected individuals (*n*(%))

| DQB1*               | Der p     |         | Der f     |            | Atopy     |           |
|---------------------|-----------|---------|-----------|------------|-----------|-----------|
|                     | +         | -       | +         | -          | +         | -         |
| 0501                | 9 (7.5)   | 10 (10) | 12 (8.7)  | 7 (8.5)    | 11 (8.5)  | 8 (8.9)   |
| 0502                | 1 (0.8)   | 1 (1)   | 1 (0.7)   | 1 (1.2)    | 2 (1.5)   | 0 (0)     |
| 0503                | 2 (1.7)   | 2 (2)   | 3 (2.2)   | 1 (1.2)    | 2 (1.5)   | 2 (2.2)   |
| 0602                | 24 (20)   | 11 (11) | 29 (21)   | 6 (7.3)    | 23 (17.7) | 12 (13.3) |
| 0603                | 4 (3.3)   | 5 (5)   | 4 (2.9)   | 5 (6.1)    | 6 (4.6)   | 3 (3.3)   |
| 0604                | 2 (1.7)   | 4 (4)   | 1 (0.7)   | 5 (6.1)    | 1 (0.7)   | 5 (5.6)   |
| 0605                | 2 (1.7)   | 0 (0)   | 1 (0.7)   | 1 (1.2)    | 2 (1.5)   | 0 (0)     |
| 0201                | 36 (30)   | 28 (28) | 45 (32.6) | 19 (23.2)  | 41 (31.5) | 23 (25.6) |
| 0301                | 21 (17.5) | 14 (14) | 24 (17.4) | 11 (13.4)  | 23 (17.7) | 12 (13.3) |
| 0302                | 9 (7.5)   | 14 (14) | 9 (6.5)   | 14 (17.1)  | 10 (7.7)  | 13 (14.4) |
| 0303                | 6 (5)     | 8 (8)   | 4 (2.9)   | 10 (12.2)* | 5 (3.8)   | 9 (10)    |
| 04                  | 4 (3.3)   | 3 (3)   | 5 (3.6)   | 2 (2.4)    | 4 (3.1)   | 3 (3.3)   |
| Totals ( <i>n</i> ) | 120       | 100     | 138       | 82         | 130       | 90        |

\*  $\chi^2 = 7.46$ ,  $P = 0.088$

class II loci (DRB1, DQA1, DQB1 and DPB1). This allowed a more precise assignment of HLA class II genotypes than most previous studies, which have largely relied upon serological typing methods [27] or have been less comprehensive in terms of loci examined [21,24].

The families selected for this study can be deemed to be representative of the population as a whole, which is reflected in the lack of significant differences in HLA

class II genotype frequencies between the study population and the general 'host' population. No significant allelic associations were seen between any DRB1, DQB1 or DPB1 alleles and the serum level of IgE, including the DRB1\*04 and 07 alleles. However, a single weak positive association was seen between DQA1\*0301 and atopy. No significant associations were detected between any DRB1, DQB1 or DPB1 alleles and skin prick reactivity

**Table 4.** HLA-DPB1 genotype frequencies and skin prick reactivity and atopy in affected and non-affected individuals (*n* (%))

| DPB1                | Der p     |         | Der f     |           | Atopy     |           |
|---------------------|-----------|---------|-----------|-----------|-----------|-----------|
|                     | +         | -       | +         | -         | +         | -         |
| 0101                | 14 (11.7) | 10 (10) | 16 (11.6) | 8 (9.8)   | 15 (11.5) | 9 (10)    |
| 0201                | 14 (11.7) | 6 (6)   | 15 (10.9) | 5 (6.1)   | 14 (10.8) | 6 (6.7)   |
| 0202                | 2 (1.7)   | 1 (1)   | 3 (2.2)   | 0 (0)     | 3 (2.3)   | 0 (0)     |
| 0301                | 11 (9.2)  | 17 (17) | 14 (10.1) | 14 (17.1) | 16 (12.3) | 12 (13.3) |
| 0401                | 53 (44.2) | 44 (44) | 63 (45.7) | 34 (41.5) | 56 (43.1) | 41 (45.6) |
| 0402                | 8 (6.7)   | 4 (4)   | 9 (6.5)   | 3 (3.7)   | 7 (5.4)   | 5 (5.6)   |
| 0501                | 5 (4.2)   | 1 (1)   | 3 (2.2)   | 3 (3.7)   | 5 (3.8)   | 1 (1.1)   |
| 0601                | 1 (0.8)   | 3 (3)   | 1 (0.7)   | 3 (3.7)   | 0 (0)     | 4 (4.4)   |
| 0901                | 2 (1.7)   | 0 (0)   | 2 (1.4)   | 0 (0)     | 2 (1.5)   | 0 (0)     |
| 1001                | 2 (1.7)   | 5 (5)   | 3 (2.2)   | 4 (4.9)   | 2 (1.5)   | 5 (5.6)   |
| 1101                | 4 (3.3)   | 2 (2)   | 3 (2.2)   | 3 (3.7)   | 3 (2.3)   | 3 (3.3)   |
| 1301                | 2 (1.7)   | 4 (4)   | 3 (2.2)   | 3 (3.7)   | 3 (2.3)   | 3 (3.3)   |
| 1601                | 0 (0)     | 2 (2)   | 1 (0.7)   | 1 (1.2)   | 1 (0.7)   | 1 (1.1)   |
| 1701                | 2 (1.7)   | 1 (1)   | 2 (1.4)   | 1 (1.2)   | 3 (2.3)   | 0 (0)     |
| Totals ( <i>n</i> ) | 120       | 100     | 138       | 82        | 130       | 90        |

**Table 5.** Haplotype sharing among affected sib pairs for skin prick reactivity to *D. pteronyssinus*, *D. farinae* and atopy

| Phenotype | Haplotypes shared |    |   | $\chi^2$ | P  |
|-----------|-------------------|----|---|----------|----|
|           | 2                 | 1  | 0 |          |    |
| Der p     | 2                 | 12 | 8 | 3.18     | NS |
| Der f     | 4                 | 14 | 8 | 5.34     | NS |
| Atopy     | 3                 | 11 | 9 | 1.39     | NS |

to either Der p or Der f. However, a significant association was seen between DQA1\*0301 and Der f skin prick test reactivity. Neither of these DQA1 associations was confirmed by affected sib-pair analysis, which did not reveal significant sharing of alleles between affected sib pairs.

The association between DQA1 and log serum IgE was significant ( $P < 0.05$ ), although weak and not confirmed by the sib-pair analysis. Most previous studies that have examined HLA associations with atopy and IgE responsiveness have not included the DQA1 locus. HLA-DQA1 alleles are in strong linkage disequilibrium with HLA-DRB1 and while any strong association would be detected by HLA-DRB typing, weaker associations may not have been evident. In addition, particular DQB1 specificities may be associated with more than one DQA1 allele, masking any DQA1 associations.

It is noteworthy that this study failed to confirm the previously reported association between the DRB1\*04 and 07 and 09 alleles (DR53 associated haplotypes) and atopy/asthma, in two studies of French white individuals [28,29], thus casting some doubt on the generalizability of these findings in white populations. In particular, the two studies by Aron *et al.* [28,29] pointed to an absence or very low incidence of DRB1\*04 alleles amongst non-atopic, non-asthmatic individuals, not seen in the present study and unusual in that DRB1\*04 is usually among the most frequent of DRB1 alleles in white populations (e.g. Howell *et al.* [41]).

Despite the lack of any evidence for a DRB1\*04 association with atopy/asthma in this study, DQA1\*0301 (found in linkage disequilibrium with DRB1\*04) was seen to be weakly associated with high serum IgE levels, in agreement with the second study of Aron *et al.* [29], which reported a significantly increased incidence of DQA1\*0301 among atopic asthmatic subjects.

The lack of any highly significant associations between particular HLA class II alleles and atopy, as evidenced by this study, is perhaps unsurprising. HLA class II

molecules are involved in the presentation of short, intracellularly processed peptides of largely exogenous origin to CD4<sup>+</sup> T cells, and are thus more likely to exhibit linkage to specific IgE responses rather than a general hyperresponsiveness to allergens. In addition, the families included in this study were selected on the basis of two or more members exhibiting sensitivity to HDM on skin prick testing. Therefore we cannot be certain that this study group is representative of atopics as a whole, which may, in part, explain the discrepancy between our results and those of Aron *et al.* [28,29].

From the results of this study it can also be concluded that HLA class II alleles appear to play a limited role in the regulation of the immune response to HDM, in the British white population at least. Only one significant association was evident, that between DQA1\*0301 and skin prick test reactivity to Der f, but there was no evidence for increased haplotype sharing between affected sib pairs. This contrasts with the evidence that the HLA DRB1, DRB3 and DRB5 genes are able to restrict recognition of HDM determinants [20,44,45]. Nevertheless, protein antigens such as Der p and Der f undoubtedly express multiple epitopes or sequence motifs that can each be presented in association with different HLA class II polymorphic molecules expressed on the surface of antigen-presenting cells. This phenomenon inevitably dilutes any association between particular HLA class II alleles and specific IgE responses to a given allergen in a population, particularly when using whole Der p/Der f allergen extracts. The use of purified allergens (e.g. Der p 1) would afford advantages for a further study of this sort. However, even then, HLA associations would only be expected if one particular epitope on the HDM allergen was immunodominant, where a restriction of response by one particular HLA allele would have a significant effect on the responsiveness to the allergen by an individual.

In conclusion, this study has failed to confirm the previously reported association between DRB1\*04 and 07 DR53-associated haplotypes and atopy. In addition we suggest that while HLA alleles do appear to restrict specific IgE responses to a number of small simple allergens (e.g. 15–19, 25, 26), it is difficult to find associations with IgE responses to more complex unpurified allergens, such as those derived from HDM, in either population- or family-based studies.

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