

HLA class II DRB1, DQB1 and DPB1 genotypic associations with peanut allergy: evidence from a family-based and case-control study

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Summary

Background Peanut is one of the most common foods provoking allergic reactions and is the most frequent cause of fatal and near-fatal food-induced anaphylaxis. However, as yet, little is known of the genetic and immunological mechanisms which underly peanut allergy.

Objective Based on findings in other allergic diseases, we have investigated whether particular human leucocyte antigen (HLA) class II genetic polymorphisms contribute to the development of peanut allergy.

Methods All individuals from 37 families each containing one or more peanut allergic individuals, plus nine unrelated patients (161 individuals in total, defined as the study group) were typed for the HLA class II DRB1, DQB1 and DPB1 loci, by PCR-based techniques. Genotype frequencies were compared with those found in 293 unrelated controls.

Results Four class II genotypes (DRB1*08 (13.7% vs 4.8%; $P_c = 0.026$), DRB1*08/12 tyr16 (22.4% vs 8.2%; $P_c = 0.021$), DQB1*04 (12.2% vs 2.7%; $P_c = 0.0026$) and DPB1*0301 (49.1 vs 22.5%; $P_c = 0.00062$)) were present at a significantly higher frequency in the study group compared with controls. Three of these genotypes (DRB1*08 (18.0%; $P_c = 0.027$), DRB1*08/12 tyr16 (24.0%; $P_c = 0.029$) and DQB1*04 (16.7%; $P_c = 0.0029$)) were also significantly increased in peanut allergic individuals compared with controls. In addition, two genotypes (DPB1*0101 and 0201) were significantly decreased in frequency in the overall study group, but not specifically in peanut allergic individuals.

Conclusion While other genetic factors may be important, results from this study indicate that HLA class II genetic polymorphism may play a role in determining susceptibility to peanut allergy.

Keywords: peanuts, allergy, genetics, HLA class II genes

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Introduction

Peanut (*Arachis hypogea*) is one of the most common foods causing acute allergic reactions in both children and adults and is, in fact, the most frequent cause of fatal and near-fatal food-induced anaphylaxis [1]. Such allergic reactions to peanut allergens, induced by ingestion or contact, develop

early in life and usually persist indefinitely [2]. The problem for the sufferer is that peanuts are particularly difficult to avoid, being present in many foodstuffs, sometimes even being disguised as other nuts [3].

While considerable advances have been made in identifying and characterizing a number of peanut allergens [4–6], as yet little is known of the genetic and immunological mechanisms which underly peanut allergy. Early identification of individuals who may be genetically at risk of developing peanut allergy is, however, an essential element in early avoidance strategies to prevent sensitization to

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peanuts and will aid the design of appropriate therapies. We hypothesize that the unique severity and persistence of peanut allergy is likely to be separately genetically determined in addition to the general genetic predisposition to atopy, although peanut allergy is much more frequent among atopic individuals.

The human leucocyte antigen (HLA) genes play a major role in regulating the immune response and particular polymorphisms of these loci play a well known role in predisposition to a large number of immunologically mediated diseases, including rheumatoid arthritis, coeliac disease, insulin dependent diabetes mellitus and multiple sclerosis [7]. HLA class I and class II molecules are encoded by the most highly polymorphic gene family in the human genome [8] and are membrane-bound glycoproteins that bind intracellularly processed antigenic peptides and present them to T cells via their cell surface T-cell receptors (TCRs). While HLA class I molecules are present on all nucleated cells (except fetal trophoblast) and platelets and function to present peptides of largely endogenous (viral) origin to CD8⁺ T-cells, which are mostly cytotoxic, HLA class II molecules are expressed on a more restricted range of cell types — including B cells, activated T cells and the monocyte/macrophage lineage and are interferon-gamma inducible. Expressed class II molecules present short peptides (12–25 aminoacids in length [9]) of largely exogenous origin to CD4⁺ T cells of mainly helper phenotype. Polymorphism of the HLA class II molecules in question influences the sequence of the peptides which may be bound and presented to CD4⁺ T cells.

A considerable body of evidence now indicates that particular HLA class II alleles also influence specific immunoglobulin E (IgE) responses to several airborne pollen, mould spore, domestic animal and house dust mite allergens [10,11] and HLA class II DR and DP molecules have been shown to restrict T-cell recognition of peanut antigens using T-cell clones derived from a peanut allergic individual [12]. In addition, peanut allergic patients may use specific TCR variable regions in the recognition of peanut allergens [13]. Based on these findings and the well-documented role of HLA class II genetic polymorphism in regulating the T cell immune response, we have investigated whether particular polymorphisms of the HLA class II DRB1, DQB1 and DPB1 loci contribute to the development of peanut allergy in a well-documented series of 37 families each containing at least one peanut allergic proband, plus an additional nine unrelated peanut allergic individuals.

Materials and methods

Study group

One hundred and sixty-one individuals were included in the

study group, which comprised 34 nuclear families each containing one peanut allergic proband, three nuclear families each containing two peanut allergic siblings (in one family, one parent was also allergic to peanuts), plus nine peanut allergic patients whose immediate family members were not available for analysis. In total, the study group of 161 individuals (hereafter referred to as the study group) contained 50 peanut allergic individuals, 34 non-peanut allergic siblings (of whom 12 were non-atopic and 22 atopic) plus non-peanut allergic parents. All individuals were of caucasian ethnic origin. The age profiles and characterization of other allergies in the child probands have been published elsewhere [14]. Briefly, only one child was mono-allergic, 51% of the group had asthma, 67% had eczema and 44% had hay fever or rhinitis. Allergy to foods other than peanuts was reported to affect 16 probands (30%), nine of which were allergic to tree nuts, six to eggs and three to fish.

Each subject (probands and family members) was evaluated by one investigator and a standard questionnaire was applied [14]. Skin tests using peanut mix 1:10 w/v (Miles Inc, Elkhart, Indiana, USA), saline 0.9% (negative control) and histamine 1% (positive control) were performed. Subjects were considered peanut allergic if history of an allergic reaction was convincing and skin-prick test was positive (weal diameter of more than 3 mm in the presence of a positive response to histamine and no response to saline). One family contained a pair of non-identical twins, both of whom were allergic to peanuts. Two other families contained non-identical twin pairs who were discordant for peanut allergy. The nine allergic patients seen without their families had a mean age of 27 years. Five were male and all were skin-prick test positive to peanut and one or more additional allergens.

Controls

A total of 293 unrelated British Caucasoid bone marrow and cadaveric renal donors previously HLA class II DRB1 and DQB1 typed in the laboratory [15; and previously unpublished data] were included as controls for the HLA class II-peanut allergy association studies. HLA class II DPB1 typing data were also available for 187 of this control series [16]. However, data on the incidence of atopy and specific allergies in this control series were unavailable.

HLA class II typing

Following DNA extraction from peripheral blood samples, HLA class II DRB1, DQB1 and DPB1 typing was performed by PCR amplification combined with sequence specific oligonucleotide probing (PCR-SSOP). PCR amplification of the second exon of the class II gene in question

was achieved using PCR primers and conditions derived from the Eleventh International Histocompatibility Workshop [17] and modified as described previously [15]. Two microlitre aliquots of PCR products were dotted onto nylon membranes and digoxigenin 5' end-labelled 18mer sequence specific oligonucleotide probes (SSOPs) were used, one probe per membrane, in a dot blot assay to determine the allelic specificities present. Oligonucleotide probes were selected from the Eleventh and Twelfth International Histocompatibility Workshop panels [17] and employed in membrane hybridization, stringency washing and enhanced chemiluminescent detection of bound probes according to established PCR-SSOP methodology [15]. Eighteen oligonucleotide probes were used for typing the DRB1 locus, 16 probes for DQB1 and 19 for DPB1. This typing system is capable of detecting all known DRB1 and DQB1 alleles, combined into 13 resolvable DRB1 (DRB1*01–14) and 7 DQB1 (DQB1*0201–06) allelic groups. All 48 DPB1 alleles or allelic groups known at the time of the study were also detectable.

Statistical analysis

HLA-DRB1, DQB1 and DPB1 genotype frequencies were scored in the peanut allergic patients, family members and in the control group. Genotype frequencies were compared between the overall study group (peanut allergic patients plus family members) and the control group, between the peanut allergic patients only and the control group and between peanut allergic patients and nonpeanut allergic siblings (both atopic and non-atopic). Significance was evaluated using the chi-square test, or Fisher's Exact test if any value in a 2×2 table was less than 5. Uncorrected probability values were examined for evidence of trends towards particular HLA genotypic associations with peanut allergy. Probability values were then corrected for the number of allelic comparisons made for each locus (i.e. 13 for DRB1, seven for DQB1 and 13 for DPB1), according to established procedures for HLA and disease association studies [18] (including studies of HLA and allergic diseases [e.g. 19, 20]), to demonstrate statistically significant genotypic associations. These analyses were performed using the University of Southampton Faculty of Medicine MED-STAT package. Relative risks [18] were calculated where a particular genotype showed a significantly increased incidence in peanut allergic individuals compared with unrelated controls.

Ethical approval

This study was approved by the local Hospital Ethics subcommittee.

Results

DRB1 typing data were obtained for all 161 individuals (including 50 with peanut allergy) in the study group. One hundred and fifty-six of these individuals were also typed for the DQB1 locus and 122 were typed for DPB1, as dictated by DNA availability. All 13 detectable DRB1 and seven detectable DQB1 allelic groups were found within the study group, but only 13 of the 48 detectable DPB1 alleles or allelic groups were present. All alleles were resolvable in all heterozygous combinations. As described in Patients and Methods, DRB1, DQB1 and DPB1 genotypic frequencies in the entire study group, in peanut allergic individuals only, in non-peanut allergic siblings (both atopic and non-atopic) and in the control group were compared. Genotypes showing significant differences in frequency (with and without correction for the numbers of comparisons made) between any of the comparison groups are summarized in Table 1.

DRB1

Of the 13 DRB1 genotypes detected, three (DRB1*08, 12 and 03) showed differing prevalences between the comparison groups as defined above. DRB1*08 was present at a significantly increased frequency in the total study group as compared with the controls (13.7% vs 4.8%; corrected probability (P_c) = 0.026). DRB1*08 was also increased in frequency among peanut allergic individuals (18.0%; P_c = 0.027; Relative Risk (RR) = 4.37) compared with controls and non-significantly among non-peanut allergic siblings (17.6%). In addition, DRB1*12 was increased in frequency in the total study group (8.7% vs 3.4%), but this increase was significant only before correcting for the number of comparisons made (P = 0.027). The same increase in DRB1*12 frequency was also seen as a non-significant trend when comparing peanut allergic individuals (6.0%) and non-peanut allergic siblings (14.7%) with controls. DRB1*08 and DRB1*12 alleles all share a TAT motif at codon 16, exon II (encoding an aromatic tyrosine residue). This motif is only found in a number of other, rare DRB1 alleles (1317, 1404, 1411 and 1415) not detected in this analysis [21]. Based on this rationale, the incidence of this sequence motif, derived from combined DRB1*08 and 12 frequencies was determined and found to be significantly increased in frequency in the overall study group (22.4%; P_c = 0.0021) and among peanut allergic individuals (24.0%; P_c = 0.029; Relative Risk (RR) = 3.54) as compared with controls (8.2%). This motif also showed a significantly increased frequency among non-peanut allergic siblings compared with controls (32.4%; P_c = 0.0027).

Finally, DRB1*03 also showed a trend towards an increased frequency among peanut allergic individuals (42.0%) compared with both controls (23.6%; P = 0.01;

Table 1. HLA class II DRB1, DQB1 and DPB1 genotype frequencies in the total study group, peanut allergic individuals, non-peanut allergic siblings and unrelated controls: significant genotypic associations with peanut allergy

Locus/allele	Total study group <i>n</i> (%)	Peanut allergic individuals <i>n</i> (%)	Non-peanut allergic siblings <i>n</i> (%)	Controls <i>n</i> (%)	<i>P/P_c</i> I	<i>P/P_c</i> II	<i>P/P_c</i> III
DRB1*	(<i>N</i> = 161)	(<i>N</i> = 50)	(<i>N</i> = 34)	(<i>N</i> = 293)			
03	46 (28.6%)	21 (42.0%)	6 (17.6%)	69 (23.6%)	NS	<i>P</i> = 0.01 <i>P_c</i> = NS	<i>P</i> = 0.033; <i>P_c</i> = NS
08	22 (13.7%)	9 (18.0%)	6 (17.6%)	14 (4.8%)	<i>P</i> = 0.0020; <i>P_c</i> = 0.026	<i>P</i> = 0.0021; <i>P_c</i> = 0.027	NS
12	14 (8.7%)	3 (6.0%)	5 (14.7%)	10 (3.4%)	<i>P</i> = 0.027 <i>P_c</i> = NS	NS	NS
08/12 (tyr16)	36 (22.4%)	12 (24.0%)	11 (32.4%)	24 (8.2%)	<i>P</i> = 0.00016 <i>P_c</i> = 0.0021	<i>P</i> = 0.0023 <i>P_c</i> = 0.029	NS
DQB1*	(<i>N</i> = 156)	(<i>N</i> = 48)	(<i>N</i> = 34)	(<i>N</i> = 293)			
0302	27 (17.3%)	7 (14.6%)	9 (26.5%)	81 (27.6%)	<i>P</i> = 0.019 <i>P_c</i> = NS	NS	NS
04	19 (12.2%)	8 (16.7%)	3 (8.8%)	8 (2.7%)	<i>P</i> = 0.00037 <i>P_c</i> = 0.0026	<i>P</i> = 0.00042 <i>P_c</i> = 0.0029	NS
DPB1*	(<i>N</i> = 122)	(<i>N</i> = 41)	(<i>N</i> = 22)	(<i>N</i> = 187)			
0101	2 (1.6%)	0 (0.0%)	1 (4.5%)	27 (14.4%)	<i>P</i> < 0.0001 <i>P_c</i> = 0.00057	<i>P</i> = 0.018 <i>P_c</i> = NS	NS
0201	3 (2.5%)	2 (4.9%)	0 (0.0%)	37 (19.8%)	<i>P</i> < 0.0001 <i>P_c</i> < 0.0001	<i>P</i> = 0.011 <i>P_c</i> = NS	NS
0301	59 (49.1%)	17 (41.5%)	8 (36.4%)	42 (22.5%)	<i>P</i> < 0.0001 <i>P_c</i> = 0.00062	<i>P</i> = 0.019 <i>P_c</i> = NS	NS
1101	0 (0.0%)	0 (0.0%)	0 (0.0%)	13 (7.0%)	<i>P</i> = 0.0071 <i>P_c</i> = NS	NS	NS

P/P_c I = uncorrected and corrected probability, total study group vs controls.

P/P_c II = uncorrected and corrected probability, peanut allergic patients vs controls.

P/P_c III = uncorrected and corrected probability, peanut allergic patients vs non-peanut allergic siblings.

N = Number of individuals typed for each locus examined.

NS = Not significant.

Probability values in bold type indicate *P* and *P_c* values for comparisons significant after correcting for numbers of comparisons made.

P_c = NS) and nonpeanut allergic siblings (17.6%; *P* = 0.033; *P_c* = NS). There was no evidence for an increased frequency of DRB1*08/12, DRB1*03 heterozygotes among either the overall study group or the peanut allergic individuals (data not shown).

DQB1

Of the seven DQB1 genotypes detected, two (DQB1*04 and 0302) showed differing prevalences between the comparison groups as defined above. DQB1*04 was significantly increased in frequency in both the total study group (12.2%; *P_c* = 0.0026) and among peanut allergic individuals (16.7%; *P_c* = 0.0029; RR = 6.79) as compared with controls (2.7%).

All DRB1*08 individuals also typed as DQB1*04 and vice versa. Conversely, a nonsignificant trend towards a decreased incidence of DQB1*0302 among the overall study group (17.3%; *P* = 0.019; *P_c* = NS) and among peanut allergic patients (14.6%; *P* = NS) as compared with the controls (27.6%), was also seen.

DPB1

Of the 13 DPB1 genotypes detected, four (DPB1*0101, 0201, 0301 and 1101) showed differing prevalences between the comparison groups as defined above. DPB1*0301 was significantly increased in frequency among the study group (49.1%; *P_c* = 0.00062) compared

with controls (22.5%). This genotype was also present at a non-significantly increased incidence among the peanut allergic individuals (41.5%; $P = 0.019$; $P_c = \text{NS}$). Conversely, two alleles (DPB1*0101 and 0201) were significantly decreased in incidence among the overall study group compared with the controls. These decreases were also seen as non-significant trends when comparing peanut allergic individuals with the control group (see Table 1 for full data). Finally, DPB1*1101 showed a non-significant trend towards a decreased incidence in the total study group and among peanut allergic patients as compared with the controls (Table 1).

None of the above DRB1, DQB1 or DPB1 genotypes which showed significant differences in frequency when comparing peanut allergic individuals with controls or non-peanut allergic siblings (independent of atopic status) showed significant differences when the peanut allergic individuals were separately compared with atopic and non-atopic non-peanut allergic siblings, due to the small numbers in each group (data not shown).

Discussion

Allergic (atopic) diseases, including peanut allergy, in children and young adults are characterized by persistent IgE responses to extrinsic protein allergens. A broad definition of atopy may include up to 40% of the population but despite many studies, debate persists over the extent and nature of genetic influences on the atopic phenotype [22]. Nevertheless, a number of epidemiological [9] and family based genetic studies [23] have clearly demonstrated that specific IgE responses to purified allergens are influenced by HLA class II genotype. These studies have concentrated on airborne pollen, mould spore, domestic animal and house dust mite allergens [10,11]. To date, the strongest and most consistent associations have been in those individuals reacting to low molecular weight minor allergenic determinants such as Amb a 5 (Ra5) of ragweed, which consists of a small peptide of 45 aminoacids [10,24]. Unlike large protein antigens, which undoubtedly express multiple epitopes, small polypeptides may express only a very small number of peptide epitopes that can each be presented to T cells in association with particular HLA class II alleles, thus making any genetic associations clearer at the population level. In addition, particular HLA class II DQB1 alleles and haplotypes have been shown to be associated with industrial asthma induced by low molecular weight compounds such as isocyanates [25]. Unlike inhalant allergy, investigations of possible genetic influences on the development of food allergies have been limited. Therefore in the present study we sought to determine whether the particular polymorphisms of the three most

polymorphic HLA class II loci — DRB1, DQB1 and DPB1 — were associated with the presence of peanut allergy in a series of families and unrelated peanut allergic individuals.

Genotypic comparisons were made in three ways. Firstly, HLA genotypes in the total study group were compared with the unrelated control series, to provide preliminary, but non-definitive evidence of over- or under-representation of particular HLA genotypes in the study group. This first method of analysis revealed that four HLA class II genotypes (DRB1*08, DRB1*08/12 tyr16, DQB1*04 and DPB1*0301) were significantly increased in frequency (in the total study group compared with the controls, while 2 genotypes (DPB1*0101 and 0201) were significantly decreased in frequency in the study group. However, such an subsequent approach cannot per se provide definitive evidence for HLA associations with peanut allergy, since skewing of HLA genotypes could arise by chance when small numbers of large families) are analysed, although such skewing was not observed in two recent HLA and allergy studies in which family groups were compared with unrelated controls [23,26]. More importantly, when comparing HLA genotypes in peanut allergic individuals alone, with the control series, three of the four HLA genotypes which were present at a significantly higher frequency in the study group (DRB1*08, DRB1*08/12 tyr16 and DQB1*04) were also significantly increased in frequency in the peanut allergic individuals compared with the unrelated controls.

None of the HLA class II associations seen in either the peanut allergic patients or total study group as compared with unrelated controls were significant when considering peanut allergic vs non-peanut allergic siblings. For a definitive sibling pair analysis, peanut mono-allergic vs non-allergic sibling pairs should be considered. However, peanut allergy is recognized as being only rarely an isolated manifestation of allergy. Re-analysis of our published data [14] suggests only 5% of our patient series are mono-allergic to peanuts, in agreement with a parallel study [27]. Thus, such an analysis could not be performed in the present study.

These preliminary findings, in particular those derived from this first case-control study of HLA and peanut allergy, indicate that allergic reactions to peanuts are at least in part under genetic control and that part of this control is likely to be modulated by HLA class II polymorphism. This conclusion is supported by a single, recently published study of a nuclear family containing four peanut-allergic siblings, which suggested that peanut allergy segregated with a single parental HLA haplotype [28]. However, functional studies will be required to demonstrate causality. Also, due to the extensive linkage disequilibrium which occurs with the Major Histocompatibility Complex (MHC), a significant role for other polymorphic MHC loci not examined in this

study, such as TNF [29], cannot formally be excluded. Therefore, our present results do not allow the precise HLA associated peanut allergy susceptibility allele, alleles or sequence motifs to be defined unequivocally.

Other factors inherent in the present study design may, in fact, have served to underestimate HLA class II or class II-related associations with peanut allergy. For example, data on the incidence of atopy and other allergic diseases among the unrelated control series were not available. A high incidence of atopy among such controls may act to mask HLA associations with atopy or specific allergies, which may only become apparent when non-atopic controls are available [20], an issue we have considered in some detail elsewhere [30]. However, due to the low prevalence of peanut allergy (1.3% of British 4-year-old children) [31], while many of our control series may have been atopic, it is unlikely that many of the controls were allergic to peanuts.

The results presented in this study are especially significant in that HLA class II genetic associations with susceptibility to peanut allergy were clearly demonstrable even when considering HLA associations with skin-prick test positivity to crude peanut allergen. Such an allergenic extract contains several identified peanut allergens (e.g. Ara h 1 and Ara h 2), each of which 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 T-cell mediated responses to a given allergen in a population. For example, a previous study from our laboratory failed to demonstrate significant HLA class II genotypic associations with house dust mite allergy as determined by skin-prick testing to crude house dust mite allergen [26], despite the fact that HLA-DRB1, DQB1 and DPB1 alleles have been shown to restrict recognition of T-cell epitopes of purified Der p 1 and Der p 2 house dust mite allergens *in vitro* [32,33]. Even when HLA class II associations with IgE responsiveness to purified allergens is investigated, strong associations are only be expected if one particular epitope on the allergen is immunodominant.

Based on these results, we hypothesize that one or more peptides derived from peanut allergens show differing affinities with particular HLA class II alleles. Allergenic peptide presentation by these class II molecules to CD4⁺ helper T cells, expressing particular TCR V gene families [13] or complementarity-determining region sequences, may lead to a Th₂ type response, triggering an IgE hypersensitive response to the peanut allergen. This is supported by studies showing that peanut allergen-specific T-cell clones isolated from the blood of peanut allergic individuals produce a Th₂ cytokine profile [12,34]. A negative HLA class II association (e.g. DPB1*0101) may reflect a low

affinity of peptide for the class II molecule in question leading to failure to trigger T cells, or peptide presentation to 'suppressor' T cells. Present results suggest that alleles or sequences within the DRB1*08, DQB1*04 haplotype are the strongest candidates for presentation of immunodominant epitopes, since DQB1*04 was found in association with DRB1*08 in all DQB1*04 individuals in the study group. In addition, residue tyr16 (mapping within the peptide binding groove of HLA-DR molecules), common to both DRB1*08 and DRB1*12 alleles is a potential candidate for a peanut allergy-associated sequence motif, since DRB1*12 also showed a nonsignificantly increased frequency in the overall study group.

In summary, based on these findings and combined with functional studies, it is very likely that once the major peanut allergens have been fully characterized and T-cell epitopes identified, it will be possible to demonstrate extremely strong associations between IgE responsiveness to these allergens and particular HLA genotypes. Furthermore, it may even be possible to identify groups who are at a high risk of having severe reactions because of exposure to particular allergenic determinants to which they are susceptible.

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References

- 1 Sampson HA, Mendelson L, Rosen JP. Fatal and near-fatal food-induced anaphylaxis in children. *N Eng J Med* 1992; 327:380–4.
- 2 Bock SA, Atkins FM. The natural history of peanut allergy. *J Allergy Clin Immunol* 1989; 83:900–4.
- 3 Keating MU, Jones RT, Worley NJ, Shively CA, Yunginger JW. Immunoassay of peanut allergens in food-processing materials and finished foods. *J Allergy Clin Immunol* 1990; 86:41–4.
- 4 Burks AW, Williams LW, Helm RM et al. Identification of a major peanut allergen Ara h I, in patients with atopic dermatitis and positive peanut challenges. *J Allergy Clin Immunol* 1991; 88:172–9.
- 5 Burks AW, Williams LW, Connaughton C et al. Identification and characterisation of a second major peanut allergen, Ara h II, with use of the sera of patients with atopic dermatitis and positive peanut challenge. *J Allergy Clin Immunol* 1992; 90: 962–9.
- 6 Burks AW, Cockrell G, Connaughton C et al. Identification of peanut agglutinin and soybean trypsin inhibitor as minor legume allergens. *Int Arch Allergy Immunol* 1994; 105:143–9.

- 7 Heard R. HLA and autoimmune disease. In: Lechler R, ed. HLA and disease. London: Academic Press, 1994: 123–51.
- 8 Bodmer JG, Marsh SGE, Albert ED et al. Nomenclature for factors of the HLA system, 1996. *Hum Immunol* 1997; 53:98–128.
- 9 Rammensee H-G, Friede T, Stevanovic S. MHC ligands and peptide motifs. First listing *Immunogenetics* 1995; 41:178–228.
- 10 Marsh DG, Blumenthal MN, Ishikawa T et al. HLA and specific immune responsiveness to allergens. In: Tsuji K, Aizawa M, Sasazuki T, eds HLA 1991. Proceedings of the Eleventh International Histocompatibility Workshop and Conference, Vol. 1. Oxford: Oxford University Press, 1992: 765–71.
- 11 Howell WM, Holgate ST. HLA genetics and allergic disease. *Thorax* 1995; 50:815–8.
- 12 Higgins JA, Lamb JR, Lake RA, O'Hehir RE. Polyclonal and clonal analysis of human CD4+ T-lymphocyte responses to nut extracts. *Immunol* 1995; 84:91–7.
- 13 Dorion BJ, Leung DYM. Selective expansion of T cells expressing V β 2 in peanut allergy. *Pediatr Allergy Immunol* 1995; 6:95–7.
- 14 Hourihane J, O'B, Dean TP, Warner JO. Peanut allergy in relation to heredity, maternal diet, and other atopic diseases: results of a questionnaire survey, skin prick testing, and food challenges. *BMJ* 1996; 313:518–21.
- 15 Howell WM, Leung ST, Jones DB et al. HLA-DRB, -DQA, and -DQB polymorphism in celiac disease and enteropathy-associated T-cell lymphoma: common features and additional risk factors for malignancy. *Hum Immunol* 1995; 43:29–37.
- 16 Sage DA, Evans PR, Howell WM. HLA-DPA1-DPB1 linkage disequilibrium in the British caucasoid population. *Tissue Antigens* 1994; 44:335–8.
- 17 Kimura A, Sasazuki T. Eleventh International Histocompatibility Workshop reference protocol for the HLA-DNA typing technique. In: Tsuji K, Aizawa M, Sasazuki T, eds HLA 1991. Proceedings of the Eleventh International Histocompatibility Workshop and Conference, Vol. 1. Oxford: Oxford University Press, 1992: 397–419.
- 18 Dyer P, Warrens A. Design and interpretation of studies of the major histocompatibility complex in disease. In: Lechler R, ed. HLA and disease. London: Academic Press, 1994: 93–121.
- 19 Cardaba B, de Pablo R, Vilches C et al. Allergy to olive pollen: T-cell response from olive allergic patients is restricted by DR7-DQ2 antigens. *Clin Exp Allergy* 26:316–22.
- 20 Aron Y, Desmazes-Dufeu N, Matran R et al. Evidence of a strong positive association between atopy and the HLA class II alleles DR4 and DR7. *Clin Exp Allergy* 26:821–8.
- 21 Marsh SGE, Bodmer JG. HLA class II region nucleotide sequences, 1995. *Tissue Antigens* 1995; 45:258–80.
- 22 Doull IJM. Maternal inheritance of atopy? *Clin Exp Allergy* 1996; 26:613–5.
- 23 Young RP, Dekker JW, Wordsworth BP et al. HLA-DR and HLA-DP genotypes and immunoglobulin E responses to common major allergens. *Clin Exp Allergy* 1994; 24:431–9.
- 24 Marsh DG, Meyers DA, Freidhoff LR et al. HLA-Dw2: a genetic marker of human immune response to short ragweed pollen allergen Ra 5. II. response after ragweed immunotherapy. *J Exp Med* 1982; 155:1452–63.
- 25 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.
- 26 Holloway JW, Doull I, Begishvili B et al. Lack of evidence of a significant association between HLA-DR, DQ and DP genotypes and atopy in families with HDM allergy. *Clin Exp Allergy* 1996; 26:1142–9.
- 27 Ewan P. Clinical study of peanut and nut allergy in 62 consecutive patients: new features and associations. *BMJ* 1996; 312:1074–8.
- 28 Donovan GR, Manioli N, Weiner JM et al. A family study of allergy: segregation with HLA but not with T-cell receptor genes. *J Allergy Clin Immunol* 1996; 97:712–3.
- 29 Wilson AG, Digiovine FS, Duff GW. Genetics of tumour necrosis factor-alpha in autoimmune, infectious and neoplastic diseases. *J Inflamm* 45:1–12.
- 30 Howell WM, Holgate ST. Human leukocyte antigen genes and allergic disease. In: Hall IP, ed. Genetics of asthma and atopy. *Monogr Allergy Basel, Karger*, 1996: Vol; 33:53–70.
- 31 Tariq SM, Stevens M, Matthews S et al. Control study of peanut and tree nut sensitisation by age of 4 years. *BMJ* 1996; 313:514–7.
- 32 Higgins JA, Thorpe CJ, Hayball JD, O'Hehir RE, Lamb JR. Overlapping T-cell epitopes in the group I allergen of *Dermaphagoides* species restricted by HLA-DP and HLA-DR molecules. *J Allergy Clin Immunol* 1994; 93:891–9.
- 33 O'Brien RM, Thomas WR, Nicholson I, Lamb JR, Tait BD. An immunogenetic analysis of the T-cell recognition of the major house dust mite allergen Der p 2: identification of high- and low-responder HLA-DQ alleles and localization of T-cell epitopes. *Immunol* 1995; 86:176–82.
- 34 de Jong E, Spanhaak S, Martens BPM et al. Food allergen (peanut) -specific TH₂ clones generated from the peripheral blood of a patient with peanut allergy. *J Allergy Clin Immunol* 1996; 98:73–81.