

Human leucocyte antigen polymorphisms in nut-allergic patients in South Wales

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Summary

Background Peanuts and tree nuts are among the most common foods provoking severe allergic reactions including fatal anaphylaxis. However, little is known of the underlying genetic and immunological mechanisms involved.

Objective Based on findings in other allergic diseases, we have investigated whether specific human leucocyte antigens (HLA) are associated with nut allergy.

Method Eighty-four patients presenting at the allergy clinic with symptoms of nut allergy were typed for the HLA Class I (HLA-A and B) and Class II (HLA-DRB1 and DQB1) loci by PCR using sequence-specific primers. Carriage frequencies were compared with 82 atopic non-nut-allergic subjects and 1798 random blood donors.

Results The frequency of HLA-B*07 (28.57%) and DRB1*11 (15.48%) was increased in the nut-allergic patients compared to the atopic controls (12.20% and 3.66%, respectively) but not when compared to the blood donors (28.86% and 10.12%). DRB1*13 and DQB1*06 were both increased in frequency in the nut allergy patients over both the atopic and blood donor controls. However, none of these increased frequencies were significant when corrected for the number of comparisons undertaken.

Conclusion At HLA '2-digit resolution' and with undifferentiated patients with nut allergy, there are no major disturbances in the frequency of HLA-A, B, DRB1 or DQB1 types. However, the difference in frequency of HLA-DRB1*11 between the nut allergy patients and the atopic controls merits further investigation as this may represent an important phenotypic relationship.

Keywords: allergy, HLA, peanut, tree nut

Submitted 1 April 2003; revised 5 November 2003; accepted 24 December 2003

Introduction

Peanuts and tree nuts are among the most common foods causing severe allergic reactions in both children and adults [1, 2] and are particularly difficult to avoid since they are found in a wide variety of foods [3].

In a population-based study of 3 year olds in the UK, the prevalence of sensitization to peanut rose from 1.3% in 1989 to 3.2% in 1995 [4].

Once sensitization has occurred nut allergy usually persists indefinitely although there is some evidence of resolution in individuals with mild disease, especially if they have low levels of peanut-specific IgE antibodies in infancy [5–7].

Identification of subjects who are predisposed to development of nut allergies would enable the implementation of avoidance strategies. Food allergies are more likely to develop during the first few years of life and children are now widely exposed to nut allergens, in the form of nut butter, at a very young age [8].

Peanut protein has also been detected in breast milk of lactating women following dietary ingestion. Thus, breast-feeding can be a route of occult exposure that may result in sensitization of 'at risk' infants [9].

Studies with house dust mite (HDM) allergens have shown that allergen avoidance, particularly in young children, may reduce the frequency and development of symptoms in allergic disease [10, 11] probably by limiting the incidence of sensitization to specific allergen. However, another recent study suggests that HDM avoidance does not significantly affect symptoms in children at high risk of developing asthma [12]. These studies highlight the conflicting evidence regarding the role of antigen exposure and development of allergic disease. Once sensitization to HDM allergen occurs, there is a high correlation with the development of symptomatic allergic disease [13].

Little is known of the genetic and immunological mechanisms underlying nut allergy. Allergy is a multifactorial disorder relating to interaction between environmental and genetic factors, which may also differ according to ethnic background [14].

Multiple twin and family analyses strongly imply a genetic basis for atopy-related traits [15] and a recent study of Danish

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twins suggested that 73% of asthma susceptibility is due to genetic factors [16].

The high concordance rate of peanut allergy seen among monozygotic twins indicates that there is a significant genetic influence on peanut allergy [17].

Human leucocyte antigen (HLA) genes play a major role in regulating the immune response and polymorphisms at these loci have a significant role in predisposition to a large number of immunologically mediated diseases.

HLA Class I alleles (A–C) are known to influence apoptosis, an important regulatory function in inflammatory responses. Polymorphisms within this region may therefore be related to the length and severity of inflammation [18].

Certain HLA Class II alleles (DR, DQ, DP) influence specific IgE responses to several airborne pollens [19–21] and HDM allergens [22, 23] and HLA-DQ7 has been positively associated with cow milk allergy [24]. HLA-DR and DP molecules have also been shown to restrict T cell recognition of peanut antigens [25].

In addition, a study of 37 families each containing one or more peanut-allergic individuals showed a significant increase in DRB1*08, DRB1*08/12 and DQB1*04 in peanut-allergic individuals compared with control subjects [26].

Based on this evidence, we have investigated whether polymorphisms within the HLA-A, B, DRB1, DQB1 loci contribute to the development of nut allergy in a clinically well-defined population.

Materials and methods

Patient group

This consisted of 84 unrelated individuals that attended the allergy clinic with a history of nut allergy (81 Caucasian and three mixed race (one Afro-Caribbean/Caucasian, one Afro-Caribbean/Arab and one Asian/Caucasian)). The same clinician evaluated each patient and a standard questionnaire was applied. Skin prick testing to a battery of common allergens, including grass pollen, HDM, tree pollen and cats, together with a positive and negative control (histamine and saline, respectively), was performed to determine atopic status. Peanut and the tree nuts Brazil, hazel nut, walnut, cashew, almond and pecan were also tested. A weal diameter of 2 mm greater than the negative control was classed as positive [27].

Blood samples for specific IgE measurement to these nuts and HLA typing were collected.

The patients' age range was 3–56 years; 40 were male and 44 female.

Controls

An 'atopic control group' comprised of 82 random subjects recruited from university and hospital staff. This group had atopy defined by a clinical history and positive skin tests to at least two common allergens. They gave no history of nut allergy, which was confirmed by negative skin prick tests (SPTs) to peanut and the six tree nuts.

Blood samples for specific IgE and HLA typing were collected from each subject. The age range was 16–61 years; 31 were male and 51 female.

In addition, 1798 HLA typed random blood donors served as a population frequency control. Data for incidence of atopy and specific allergies in this control group were unavailable.

HLA typing

HLA-A, B, DRB1 and DQB1 typing was performed by the PCR using sequence-specific primers at the 'split specificity/2-digit' level as a minimum [28].

Specific IgE measurement

Specific IgE was determined using the Pharmacia Uni-Cap system.

Statistical analysis

The HLA-A, B, DRB1 and DQB1 carriage frequencies of the patient and atopic control groups were compared with each other and with the random blood donors.

Frequency comparisons were made using Woolf–Haldane analysis [29]; *P*-values were corrected for the number of comparisons made using Edwards' [30] method.

Ethical approval

Ethical approval for this study was obtained from the local ethics committee and both patient and 'atopic controls' gave specific informed consent.

Results

The skin prick testing showed 80 subjects were atopic in terms of skin test reactivity to a range of common antigens, of these 73 gave a positive SPT to grass and 34 to birch pollen. There was no significant association between birch pollen sensitivity and sensitivity to tree nuts. The study group contained only two patients who were sensitized to peanut and not tree nuts, both of these subjects were children, 63 were sensitized to peanut and tree nuts and 19 to tree nuts only. Of the two patients in whom only sensitivity to peanut was observed, one gave a negative SPT to all the common test allergens but gave a positive SPT to peanut.

The age of onset of the nut allergy was between 1 and 51 years and symptoms varied from mild (urticaria) to severe (anaphylaxis).

Specific IgE to peanut and/or tree nut was detected in each test patient. No specific IgE to nut protein was detected in any of the atopic control group.

HLA-A, B, DRB1 and DQB1 typing were obtained for all 166 individuals in the study. The results of all significant differences in allele group frequencies between the patients and the control groups are shown in Table 1.

HLA-B*07 was low in the atopic controls (12.20%) compared to the other two groups' patients (28.57%, *P* < 0.02); blood donor controls (26.86%, *P* < 0.01).

Table 1. Significant allele group frequencies in nut allergic patients

HLA	Nut allergy patients (<i>n</i> = 84) (%)	Atopic controls (<i>n</i> = 82) (%)	<i>P</i> -value, corrected <i>P</i> in parentheses	Blood donor controls (<i>n</i> = 1798) (%)	<i>P</i> -value, corrected <i>P</i> in parentheses
B*07	28.57	12.20	<0.02 (= 0.53)	26.86	NS
DRB1*11	15.48	3.66	<0.02 (= 0.70)	10.12	NS
DRB1*13	28.57	19.51	NS	18.74	<0.05 (= 0.82)
DQB1*06	55.95	41.46	NS	40.55	<0.01 (= 0.37)

All frequency comparisons were non-significant when corrected for the 58 comparisons made. HLA, human leucocyte antigens; NS, non-significant.

DRB1*11 was high in the patients (15.48%) vs. the atopic controls (3.66%, $P < 0.02$). The HLA-DRB1*11 frequency in the blood donors was 10.12%. Frequency comparisons between the blood donors and the patients and atopic controls did not reach statistical significance.

DRB1*13 was high in the patients (28.57%) vs. the blood donor controls (18.74%, $P < 0.05$). The DRB1*13 frequency in the atopic controls (19.51%) was very similar to the blood donor controls. The HLA-DRB1*13 frequency comparison between the patients and the atopic controls was not significant ($P > 0.05$).

Similarly, DQB1*06 was high in the patients (55.95%) vs. the blood donor controls (40.55%, $P < 0.01$) and its frequency in the atopic controls (41.46%) was very similar to the blood donor controls. The DQB1*06 frequency comparison between the patients and the atopic controls was not significantly different.

However, all frequency comparisons were non-significant (P corrected > 0.05) when their P -values were corrected for the 58 comparisons made Table 1.

Discussion

This study identifies HLA-B*07 and DRB1*11 as showing a higher frequency in the nut-allergic patient group compared to the atopic control group.

A number of previous studies have suggested genetic influences for the development of allergies [31, 32].

The role of HLA Class I and II alleles in susceptibility to allergic disease is the subject of considerable debate and several authors have identified HLA genotypes related to the development of allergy. However, it has been suggested that many earlier studies showing an association with Class II genes were related to low molecular weight allergens. As these express few epitopes, associations with genotype are easier to identify [33, 34].

Associations with Class I alleles and atopy are less clear, as antigen processing and presentation via Class I molecules have no direct implication for the recognition of allergenic epitopes. However, a limited number of studies looking at HLA-B alleles and atopy have been performed; the results of these are often inconclusive. A study by Kruszewski et al. [35] suggested that HLA-Bw53 had a high frequency in atopics and a 2002 study by Hetherington et al. [36] indicates that HLA-B57 was significantly higher in white males with sensitivity to the antiretroviral drug abacavir.

DRB1*11 associations with allergy to HDM [37] and various drugs [38] have recently been reported. Allergens

associated with peanut and tree nuts have multiple epitopes thus strong association with HLA Class II alleles are considerably more difficult to establish. This is due to the larger number of antigenic peptides that can be derived from such allergens; this in turn means that allergenic components may be bound by a number of Class II molecules during processing and presentation. However, in our study the nut allergy group shows a markedly high expression of this allele compared to the atopic controls (uncorrected $P < 0.02$).

The 82 atopic control subjects showed a significantly lower incidence of DRB1*11 (3.66%) compared to nut allergy patients. This comparison did not achieve significance when compared with blood donor controls. This suggests that this allele is reduced in those with atopy in the absence of nut allergy.

DQB1*06 was more frequent in nut allergy sufferers than either the atopic controls or the blood donor controls although the difference only reached significance with the blood donor controls. A similar picture was seen with DRB1*13, which is in linkage disequilibrium with DQB1*06, suggesting that if there is a relationship with HLA Class II and nut allergy in our population, it is likely to involve this haplotype.

Environmental factors are also very important to the development of nut allergy. China has a high peanut consumption rate, similar to that in the USA, yet the incidence of peanut allergy is extremely low. In China peanuts are commonly fried or boiled whereas in the USA they are dry roasted, which apparently increases the allergenic property of peanut protein [39]. Peanut allergy in the children of Chinese immigrants to the USA and the children of native-born Americans has similar prevalence [40].

Gene-environment interaction may manifest in differential exposure risk based on an individual's genotype or exposure. Most allergic diseases require an environmental inciting agent to be manifest. The identification of groups of individuals likely to be affected by exposure owing to their genetic susceptibility is paramount to early intervention therapy and therefore potentially of major significance to public health and health economics. The recognition of susceptible genotypes before the manifestation of symptomatic disease represents an important tool in reducing the prevalence of atopic disease by targeted intervention such as allergen avoidance.

The significant increase in the prevalence of nut allergy over the past decades cannot be explained by changes in genetic predisposition since they have occurred too quickly. It is probable that various pre-existing genetic factors interacting with a dramatically changing environment, including diet, have rendered a large percentage of the population susceptible to this disorder.

Because of the multifactorial nature of atopy gene–gene interactions have to be taken into consideration, for example, when both the genes encoding pro-inflammatory cytokines (i.e. IL-13) as well as their receptors (IL-4R α) and/or genes involved in signal transduction (STAT6) bear polymorphisms associated with disease. Recently, the first studies have demonstrated the feasibility and importance of gene–gene interaction in atopy [41].

Interestingly HLA-B*07 was also high in the nut-allergic group. Class I alleles are known to influence apoptosis and this is an important regulatory function of inflammatory responses. The higher frequency of this allele in the patient group may be suggestive of an association with disease severity in nut allergy.

Our study suggests that the presence of HLA-B*07 and HLA-DRB1*11 may be important predisposing factors in nut allergy. The low prevalence of HLA-DRB1*11 in the atopic control group may suggest that expression of this particular allele may be related to specific aspects of nut allergy rather than atopy. A study by Faux et al. [42] of patients with bee and wasp venom sensitivity also showed an increase in DRB1*11 which was not significant when corrected for multiple comparisons. Since this group of patients can suffer systemic responses it is possible that this phenotype may be related to such responses.

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