

HLA-DR and HLA-DP genotypes and immunoglobulin E responses to common major allergens

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Summary

In order to test for human histocompatibility leucocyte antigens (HLA) class II restriction of IgE responses, 431 subjects from 83 families were genotyped at the HLA-DR and HLA-DP loci and serotyped for IgE responses to six major allergens from common aero-allergen sources. A possible excess of HLA-DR1 was found in subjects who were responsive to *Fel d* I compared with those who were not (Odds Ratio (OR) = 2, $P = 0.002$), and a possible excess of HLA-DR4 was found in subjects responsive to *Alt a* I (OR = 1.9, $P = 0.006$). Increased sharing of HLA-DR/DP haplotypes was seen in sibling pairs responding to both allergens. *Der p* I, *Der p* II, *Phl p* V and *Can f* I were not associated with any definite excess of HLA-DR alleles. No significant correlations were seen with HLA-DP genotype and reactivity to any of the allergens. The results suggest class II HLA restriction is insufficient to account for individual differences in reactivity to common allergens.

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Introduction

Atopy is characterized by immunoglobulin E (IgE) responses to ubiquitous respirable proteins. In Europe, these allergens arise from house dust mite (*Dermatophagoides* spp), grass and tree pollens, cat and dog, and moulds such as *Alternaria* and *Aspergillus* spp. After initial sensitization in predisposed individuals, re-exposure to allergen results in IgE-mediated mast-cell degranulation, airway inflammation, and symptoms of wheeze and rhinitis. As a result of atopic sensitization approximately 10% of children and young adults will suffer asthma [1,2], and a larger proportion of young adults will suffer seasonal rhinitis.

In the general population and within families different individuals react to different antigens [3]. The risk of clinical disease is related to particular antigen responses, so that positive skin tests to house dust mite or to mould antigens carry a significant increase of risk of bronchial hyperresponsiveness and asthma, compared with reactivity to grass pollens [4,5]. It is therefore of interest to test if

there is genetic control of the specificity of IgE responses in atopic subjects.

A likely candidate for controlling specific IgE responses is the major histocompatibility complex (MHC). This region includes genes coding for human leucocyte antigen class II molecules (HLA-DR, DQ and DP) which are involved in the recognition and presentation of exogenous peptides.

HLA restriction of the IgE response was first noted by Levine *et al.* [6], who found an association between HLA haplotypes and IgE responses to antigen E derived from ragweed allergen (*Ambrosia artemisiifolia*). This association has been subsequently found to be due to restriction of the response to a minor component of ragweed antigen (*Amb a* V) by HLA-DR2 [7]. Other studies have claimed HLA-DR and DQ associations with IgE responses to other antigens [8–12], but often have not been reproduced.

In order to test if HLA class II gene products have a general influence on the ability to react to common allergens, we have genotyped for HLA-DR and HLA-DP in a large sample of atopic subjects from the British population. The subjects have been tested for IgE responses to the most common British major allergens.

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In addition to searching for population associations with particular HLA class II alleles, we have used sib-pair tests for genetic linkage to increase the power of the study. We have also used regressive models to control for an individual's reactions to more than one antigen.

Materials and methods

Seventy-six nuclear and seven extended families were recruited through subjects attending allergy and asthma clinics or following responses to media appeal. The families contained 431 subjects.

HLA-DR antigen frequencies in controls were estimated from 297 unrelated individuals from the south of England, typed by the Oxfordshire regional tissue typing laboratory. HLA-DP antigen frequencies of controls were estimated by DNA typing 312 unrelated British individuals randomly selected from available DNA pools (98 subjects) or from the Haematology Laboratory at the John Radcliffe Hospital, Oxford (214 subjects).

The purified allergen molecules used in the determination of specific IgE were the following: *Der p* I (25.4 kDa) and *Der p* II (14.1 kDa) from the house dust mite *Dermatophagoides pteronyssinus*; the allergens were puri-

fied physico-chemically as previously described [13]. *Alt a* I (28 kDa) from the mould *Alternaria alternata* was purified as described [14]. *Can f* (25 kDa) from the dog *Canis familiaris* was purified by immunoaffinity chromatography [15]. *Fel d* I (18 kDa) from the cat *Felis domesticus* was purified by immuno-affinity chromatography [16]. *Phl p* V (30 kDa) from Timothy grass, *Phleum pratense* was purified physico-chemically as described [17].

The subjects were tested for IgE specific for purified allergens by a chemiluminescence assay derived from the Magic Lite System (Ciba Corning Diagnostics, Walpole, Ma., USA and ALK, Hørsholm, Denmark) [18,19]. The allergens were covalently coupled to microscopic paramagnetic particles. These were used for detection of bound IgE as revealed in a luminometer (Magic Lite Analyzer, Ciba Corning Diagnostics) by a monoclonal IgE (ALK) coupled to a chemiluminescent acridium ester. A positive response is equivalent to a RAST score of 1 (Pharmacia Cap System, Pharmacia, Uppsala, Sweden). Because of lack of serum, full IgE serotyping was not carried out on three subjects.

HLA-DRB1 and HLA-DPB1 allele frequencies from the subjects were determined by sequence-specific oligo-

Table 1. HLA-DR allele frequencies and specific IgE reactions to major antigenic determinants of timothy grass and *alternaria*

HLA-DR % (n)	IgE reactions				Controls (%)
	<i>Phl p</i> V		<i>Alt a</i> I		
	—	+	—	+	
Untyped	2 (10)	0 (0)	1 (10)	2 (2)	
HLA-DR*1*	10 (66)	10 (21)	10 (74)	12 (13)	12
HLA-DR*2	17 (112)	12 (24)	17 (125)	10 (11)	18
HLA-DR*3	17 (111)	17 (34)	18 (132)	12 (13)	14
HLA-DR*4	15 (100)	23 (46)§	16 (118)	26 (28)†	18
HLA-DR*7	10 (68)	12 (25)	11 (79)	13 (14)	11
HLA-DR*8	1 (7)	2 (4)	1 (10)	1 (1)	2
HLA-DR*9	2 (13)	3 (5)	2 (16)	2 (2)	1
HLA-DR*10	0 (2)	0 (1)	0 (2)	1 (1)	0
HLA-DR*11	8 (54)	7 (13)	9 (64)	3 (3)	10
HLA-DR*12	1 (8)	1 (2)	1 (10)	0 (0)	1
HLA-DR*13	10 (66)	10 (19)	9 (71)	13 (14)	10
HLA-DR*14	4 (26)	1 (2)	3 (25)	3 (3)	3
Others¶	2 (13)	2 (4)	2 (16)	1 (1)	
Totals	656	200	752	106	594

§ $\chi^2 = 6.5$, $P = 0.01$, OR = 1.6; † $\chi^2 = 7.6$, $P = 0.006$, OR = 1.9; * Including HLA-DRB1*0103; ¶ Including DRw17 & DRw18.

Table 2. HLA-DR allele frequencies and specific IgE reactions to major antigenic determinants of house dust mite

HLA-DR % (n)	IgE reactions			
	Der p I		Der p II	
	-	+	-	+
Untyped	1 (8)	2 (4)	1 (8)	2 (4)
HLA-DR*1*	10 (63)	10 (24)	10 (53)	10 (32)
HLA-DR*2	16 (96)	16 (40)	17 (92)	14 (44)
HLA-DR*3	17 (105)	16 (40)	16 (86)	19 (59)
HLA-DR*4	17 (104)	17 (42)	16 (90)	18 (56)
HLA-DR*7	11 (68)	10 (25)	12 (64)	9 (29)
HLA-DR*8	1 (6)	2 (5)	1 (4)	2 (7)
HLA-DR*9	2 (14)	2 (4)	3 (14)	1 (4)
HLA-DR*10	0 (2)	0 (1)	0 (2)	0 (1)
HLA-DR*11	7 (44)	9 (23)	7 (38)	9 (29)
HLA-DR*12	1 (7)	1 (3)	1 (7)	1 (3)
HLA-DR*13	10 (60)	10 (25)	11 (58)	9 (27)
HLA-DR*14	3 (20)	3 (8)	4 (21)	2 (7)
Others¶	1 (9)	3 (7)	1 (7)	2 (7)
Totals	606	252	548	308

* Including HLA-DRB1*0103; ¶ Including DRw17 and DRw18.

nucleotide typing of PCR products as previously described [20,21]. HLA-DR types included HLA-DR*1-14. Sub-types were recognized for HLA-DR*1 (HLA-DR*0103), HLA-DR*3 (HLA-DR*0301=HLA-DRw17 and HLA-DR*0302=HLA-DRw18), HLA-DR*5 (HLA-DR*11 and HLA-DR*12) and HLA-DR*6 (HLA-DR*13 and HLA-DR*14) (Tables 1-3). Testing was carried out for 18 HLA-DP alleles (Tables 4-6): these include HLA-DP*0101, 0201, 0202, 0301, 0401, 0402, 0501, 0601, 0801, 0901, 1001, 1101, 1301, 1401, 1501, 1601, 1701 and 1801 [22].

HLA-DR controls ($n=297$) from the local population were typed by the standard NIH microlymphocytotoxicity assay for 12 HLA-DR specificities.

The typing of 18 recognized HLA-DPB1 alleles [22] was performed on 312 controls by restriction enzyme analysis of the DPB1 locus second exon [23]. Ninety-eight controls were also typed by SSO blotting, to ensure concordance between typing methods. There was complete agreement between the two methods.

The results were inspected for differences in HLA-DR and HLA-DP allele frequencies between the subjects with IgE responses to the major antigenic determinants and non-responders. Possible differences were assessed by chi-square analysis of contingency tables.

In order to control for individual reactions to more than one antigen, logistic regression analysis was carried out with the presence or absence of a given HLA-DR genotype as the dependent variable, and the six possible IgE serotypes as independent (predictor) variables. The regressions were carried out stepwise with the logistic regression model of the SPSS computer program [24]. All variables were included in the initial model, with removal of the least significant independent variable from the model at each step until only significant effects remained.

Genetic linkage at the HLA loci was tested in sibling pairs with positive IgE responses to individual antigens. To increase the available information at these loci HLA-DR/DP haplotypes were assigned for each subject. Haplotype sharing was counted only if all four parental haplotypes were unambiguously identified. For non-linkage the expected sharing of haplotypes by a sib-pair was assumed to be 2:25% 1:50% 0:25%. Sib-pairs from multiple sibships were treated as independent [25]. An excess sharing from that expected was tested for by chi-square estimation.

Results

There was no difference in the frequency of HLA-DR alleles in the study subjects and those in the control

Table 3. HLA-DR allele frequencies and specific IgE reactions to major antigenic determinants of dog and cat danders

HLA-DR % (n)	IgE reactions			
	<i>Can f 1</i>		<i>Fel d 1</i>	
	–	+	–	+
Untyped	1 (10)	2 (2)	1 (10)	0 (0)
HLA-DR*1*	10 (77)	10 (10)	9 (58)	16 (29)§
HLA-DR*2	16 (122)	14 (14)	17 (112)	13 (24)
HLA-DR*3	17 (127)	18 (18)	16 (111)	19 (34)
HLA-DR*4	17 (127)	19 (19)	18 (120)	15 (26)
HLA-DR*7	11 (86)	7 (7)	10 (69)	13 (24)
HLA-DR*8	1 (9)	2 (2)	1 (10)	1 (1)
HLA-DR*9	2 (15)	3 (3)	2 (15)	2 (3)
HLA-DR*10	0 (2)	1 (1)	0 (3)	0 (0)
HLA-DR*11	8 (62)	5 (5)	8 (57)	6 (10)
HLA-DR*12	1 (10)	0 (0)	1 (9)	1 (1)
HLA-DR*13	9 (71)	14 (14)	10 (68)	10 (17)
HLA-DR*14	4 (27)	1 (1)	4 (24)	2 (4)
Others¶	2 (15)	2 (2)	2 (12)	3 (5)
Totals	760	98	678	178

§ $\chi^2=9.2$, $P=0.002$, OR=2; * Including HLA-DRB1*0103;

¶ Including DRw17 and DRw18.

population ($\chi^2=11.5$, 11 d.f., $P=0.4$). Similarly, there was no difference in the frequency of the five most common HLA-DP alleles (HLA-DPB*0101, 0201, 0301, 0401 and 0402) between subjects and controls ($\chi^2=4.9$, 4 d.f., $P=0.3$). For simplicity, comparisons were therefore made between reactive and non-reactive subjects in the test population.

Fifty-three subjects were found to have positive IgE responses to *Alt a I*, the principal allergen from the mould *Alternaria alternata*. An excess of HLA-DR4 alleles was found in subjects responsive to *Alt a I* (Table 1) (26% vs 16%, $\chi^2=7.6$, OR=1.9, $P=0.006$). This was associated with increased sharing of HLA-DR alleles in sibling pairs reacting to this antigen (Table 7) ($\chi^2=4.0$, $P=0.05$). There was no difference in the frequency of HLA-DP alleles between responders and non-responders or controls (Table 4).

One hundred subjects reacted to *Phl p V*, the major allergen of Timothy grass pollen. A weak association was seen between an IgE response to this antigen and the frequency of HLA-DR4 alleles (Table 1): the antigen frequency for *Phl p V* positive subjects was 23%, compared with 15% in non-responders. The odds ratio was 1.6, and the $\chi^2=6.5$ ($P=0.01$). The significance of this apparent association is difficult to assess, particularly as

no increase in sharing of HLA class II haplotypes was seen in affected sibling pairs (Table 7). The small increase in the frequency of HLA-DPB*0201 in responders (13% vs 8%, OR=1.9, $\chi^2=4.8$, $P=0.03$) (Table 4) was attributable to chance.

Thirty-five subjects reacted both to *Alt a I* and *Phl p V*, raising the possibility that the HLA-DR4 association might be to only one of these two antigens. To test this hypothesis a backwards stepwise logistic regression was carried out with HLA-DR4 genotype as the dependent variable, and serotypes to *Alt a I* and *Phl p V* as independent. The results of the regression indicated that HLA-DR4 is significantly associated only with the *Alt a I* serotype (Table 8).

One hundred and twenty-six subjects had a positive IgE response to the house dust mite major allergen *Der p I* and 154 subjects had responded to *Der p II*. One hundred and seven subjects responded to both allergens. There was no excess of any HLA-DR or HLA-DP allele when these individuals were compared with those not responding to the respective allergen, or when compared with unrelated controls (Table 2 and Table 5). Counting of haplotypes in sib-pairs with positive responses showed no sharing beyond that expected (Table 7).

In view of the recognized *in vitro* HLA-DR3 restriction

Table 4. HLA-DP allele frequencies and specific IgE reactions to major antigenic determinants of timothy grass and *alternaria*

HLA-DPB1* % (n)	IgE reactions				Controls
	<i>Phl p V</i>		<i>Alt a I</i>		
	—	+	—	+	
Untyped	4 (25)	2 (3)	3 (25)	5 (5)	
0101	9 (56)	8 (15)	8 (62)	8 (9)	6 (40)
0201	13 (83)	8 (16)	11 (85)	13 (14)	10 (60)
0202	2 (13)	1 (2)	2 (14)	1 (1)	1 (4)
0301	9 (57)	11 (21)	10 (72)	6 (6)	10 (61)
0401	44 (287)	52 (104)	45 (339)	49 (52)	44 (277)
0402	10 (68)	10 (20)	10 (76)	11 (12)	13 (79)
0501	2 (11)	1 (1)	1 (11)	1 (1)	2 (10)
0601	2 (10)	2 (3)	2 (13)	0 (0)	4 (22)
0901	1 (6)	1 (1)	1 (7)	0 (0)	2 (13)
1001	1 (8)	3 (5)	1 (10)	3 (3)	3 (17)
1101	3 (18)	3 (5)	3 (21)	2 (2)	2 (13)
1301	1 (8)	1 (2)	1 (10)	0 (0)	1 (9)
Others¶	1 (6)	1 (2)	1 (7)	1 (1)	3 (19)
Totals	656	200	752	106	624

¶ Including 0801 1401 1501 1601 1701 1801.

Table 5. HLA-DP allele frequencies and specific IgE reactions to major antigenic determinants of house dust mite

HLA-DPB1* % (n)	IgE reactions			
	<i>Der p I</i>		<i>Der p II</i>	
	-	+	-	+
Untyped	4 (22)	3 (8)	4 (23)	2 (5)
0101	9 (53)	7 (18)	9 (49)	7 (22)
0201	12 (71)	11 (28)	11 (60)	13 (39)
0202	1 (7)	3 (8)	1 (7)	3 (8)
0301	9 (57)	8 (21)	9 (47)	10 (31)
0401	45 (272)	47 (119)	44 (243)	48 (148)
0402	11 (64)	10 (24)	11 (61)	9 (27)
0501	2 (11)	0 (1)	2 (9)	1 (3)
0601	1 (7)	2 (6)	2 (9)	1 (4)
0901	1 (6)	0 (1)	1 (5)	1 (2)
1001	1 (8)	2 (5)	1 (8)	2 (5)
1101	3 (17)	2 (6)	3 (14)	3 (9)
1301	1 (6)	2 (4)	1 (6)	1 (4)
Others¶	1 (5)	1 (3)	1 (7)	0 (1)
Totals	606	252	548	308

¶ Including 0801 1401 1501 1601 1701 1801.

Table 6. HLA-DP allele frequencies and specific IgE reactions to major antigenic determinants of dog and cat danders

HLA-DPB1* % (n)	IgE reactions			
	<i>Can f</i> 1		<i>Fel d</i> 1	
	-	+	-	+
Untyped	4 (27)	3 (3)	4 (27)	1 (1)
0101	9 (65)	6 (6)	8 (54)	10 (17)
0201	11 (84)	15 (15)	11 (77)	12 (22)
0202	2 (14)	1 (1)	2 (12)	2 (3)
0301	10 (73)	5 (5)	9 (64)	8 (14)
0401	45 (343)	48 (49)	46 (309)	46 (82)
0402	10 (78)	10 (10)	9 (63)	14 (25)
0501	1 (11)	1 (1)	2 (11)	1 (1)
0601	2 (13)	0 (0)	2 (11)	1 (2)
0901	1 (6)	1 (1)	1 (5)	1 (2)
1001	1 (9)	4 (4)	2 (11)	1 (2)
1101	3 (21)	2 (2)	3 (18)	3 (5)
1301	1 (9)	1 (1)	1 (9)	1 (1)
Others¶	1 (7)	1 (1)	1 (7)	1 (1)
Totals	760	98	678	178

¶ Including 0801 1401 1501 1601 1701 1801.

Table 7. Sharing of HLA-DR/DP haplotypes by sibling pairs affected with IgE responses to specific allergens

Phenotype	Alleles shared			χ^2	P
	2	1	0		
<i>Phl p</i> V	3	2	4	3.00	NS
<i>Alt a</i> I	4	2	0	6.00	0.05
<i>Der p</i> I	4	7	3	0.14	NS
<i>Der p</i> II	6	8	9	2.91	NS
<i>Fel d</i> 1	6	1	3	8.20	0.02
<i>Can f</i> 1	1	1	1	0.33	NS

of T-cell proliferation to house dust mite by antigen presenting cells [26], a logistic regression was carried out with HLA-DR3 as the dependent variable, and the six IgE serotypes as independent variables (Table 8). The regression uncovered a weak positive association with HLA-DR3 and *Der p* II ($P=0.03$), which has been obscured by a negative association between HLA-DR3 and *Der p* I ($P=0.08$).

Eighty-nine subjects had reacted to the major cat allergen, *Fel d* 1 (Table 3). A positive response to this antigen was associated with an excess of HLA-DR 1. (16% vs 9%, $\chi^2=9.2$, OR=2, $P=0.002$). This was associated with an increase in sib-pair sharing of HLA-DR/DP haplotype ($P=0.02$) (Table 7). A logistic regression with HLA-DR1 genotype as the dependent variable shows reactivity to other genotypes was not confounding this association (Table 8). There was no difference in the frequency of HLA-DP alleles between responders and non-responders or controls (Table 6).

Only 49 subjects reacted to the major dog allergen *Can f* I. There was no difference in the allele frequencies for HLA-DR or DP (Table 3 and Table 6), and no increase in allele sharing by affected sibling-pairs (Table 7).

Regressions to other common HLA-DR genotypes (HLA-DR2, HLA-DR7 and HLA-DR13) found no other significant associations.

Discussion

The results show only weak associations between HLA-DR allele frequencies and IgE responses to common allergens, namely HLA-DR4 with *Alt a* I and *Phl p* V, HLA-DR1 and *Fel d* I, and HLA-DR3 with *Der p* II.

Table 8. Stepwise logistic regression analysis¶

Dependent variable	Independent variables	B (Standard error)	Wald test χ^2	P
HLA-DR4	<i>Alt a</i> I	0.667 (0.243)	7.5	0.006
	Constant	-2.33 (0.299)	61.0	0.000
HLA-DR3	<i>Der p</i> I	-0.470 (0.267)	3.1	0.078
	<i>Der p</i> II	0.520 (0.245)	4.5	0.034
	Constant	-2.33 (0.299)	31.3	0.000
HLA-DR1	<i>Fel d</i> I	0.613 (0.263)	5.44	0.020
	Constant	-3.085 (0.36)	72.3	0.000

¶ All IgE responses included in the initial model. Only significant outcomes after backwards stepwise removal of non-significant effects shown.

Of these associations, that of *Alt a* I with HLA-DR4 and of *Fel d* I with HLA-DR1 are supported by a finding of excess sharing of a HLA haplotype in affected sibling pairs. Regression analysis shows the apparent association of *Phl p* V with HLA-DR4 is due to the presence of many individuals who have reacted with an IgE response both to *Alt a* I and *Phl p* V. The association of HLA-DR1 and *Fel d* I is the strongest statistically, and is significant even taking the multiple comparisons into account.

The present study is a large one, involving HLA-DR and DP typing of over 700 subjects and controls. Both subjects and the controls have come from the same relatively homogeneous population. In the United Kingdom and Europe, allergens other than *Bet v* I and those tested for here are uncommon causes of sensitization and IgE-mediated allergy.

The results suggest that HLA-DR alleles do modify the ability to mount an IgE response to particular antigens. However, the odds ratio for the association of *Alt a* I with HLA-DR4 was only 1.9, and that of *Fel d* I with HLA-DR1 was 2.0.

Of the results of the many previous investigations, only that of the minor ragweed allergen *Amb a* V (molecular weight 5000) and HLA-DR2 has been consistently confirmed [6,7,27]. Other suggested associations are of the rye grass antigens *Lol p* I, *Lol p* II and *Lol p* III with HLA-DR3 (in the same 53 allergic subjects) [8,9], American feverfew (*Parthenium hysterophorus*) and HLA-DR3 in 22 subjects from the Indian sub-continent [12], the IgE response to *Bet v* I, the major allergen of birch pollen, and HLA-DR3 in 37 European subjects [10], and an HLA-DR5 association with another ragweed antigen *Amb a* VI in 38 subjects [11].

Other authors have reported negative associations with particular allergens. These include HLA-DR4 and IgE

responses to mountain cedar pollen ($n=37$ subjects) [28] and HLA-DR4 and melittin (from bee venom) ($n=22$ subjects) [29]. Non-responsiveness to Japanese cedar pollen may be associated with HLA-DQw8 [30,31].

There is to date no confirmation of many of these results, and the number of subjects has generally not been high enough to establish an unequivocal HLA association. In addition, there has not been any recognition of the problems of reactivity to multiple allergens: significant relationships between HLA-DR alleles and five antigens (*Amb a* V, *Lol p* I, *Lol p* II, *Lol p* III and *Amb a* 6) have been claimed from the same pool of approximately 200 subjects [7-9,11]. Many of these subjects have had ragweed immunotherapy [11], so that the importance of any finding is further obscured.

The present study is the first to investigate HLA-DP alleles and reactivity to common allergens. As no definite correlation was found between any antigen response and HLA-DP genotypes with substantial numbers of subjects, HLA-DP genes are unlikely to have a major role in restricting IgE responses to these allergens. The study gives no direct information about HLA-DQ alleles and IgE responses. The HLA-DQ region is in strong linkage disequilibrium with HLA-DR, so that significant HLA-DQ associations would have been detected by HLA-DR typing, or by the sib-pair analysis with the HLA-DR/DP haplotype. In the absence of strong associations, the subjects have not been HLA-DQ typed. Nevertheless weak associations may be forthcoming if HLA-DQ is studied subsequently.

Thus, although it is possible a greater degree of HLA restriction will be evident with epitope-specific molecules derived from recombinant allergens, the relative risk associated with HLA-DR genotype and IgE responses to whole allergens does not suggest that the HLA class II loci

are of clinical importance in allergic IgE reactions. It is therefore likely that environmental factors [32,33] or other loci such as T-cell receptor genes may be of greater relevance in determining an individual's susceptibility to specific allergens.

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