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HLA antigens in Greek children with allergic bronchial asthma

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Abstract: The aim of our study was to investigate the genetic linkage between mite allergic bronchial asthma and HLA class I and II antigens and haplotypes. Sixty Greek children with allergic bronchial asthma due to mite sensitivity (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*) and their family members were typed for HLA class I and II antigens (total 263 subjects). One hundred and twenty-five healthy, unrelated Greek children without medical history of atopy were also typed as control group. Major histocompatibility complex class I and II gene analysis revealed that only HLA-DRB1*04 and HLA-DQA1*0301 alleles are possibly important factors in the development of atopic asthma in Greek children with sensitivity to mites. No significant differences among the HLA-DRB1*04 subtypes have been established. Transmission disequilibrium test revealed that no specific HLA-A, -B, -DRB1, -DQA1 and -DQB1 alleles were transmitted preferentially to the affected children. HLA-DQB1*0301-4 alleles were associated with high levels of total serum immunoglobulin E in affected children. The study of the HLA haplotypes failed to demonstrate any significant association between any extended or natural selection haplotype and mite allergic bronchial asthma in Greek children.

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Allergic asthma is considered a multifactor disease; clinical manifestations depend strongly on a specific immunoglobulin E (IgE) response to environmental allergens, most often mites (1). House dust mites (HDMs), *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, are the most important sources of indoor allergens causing allergic diseases in Greece (2). The human major histocompatibility complex (MHC) is known to contribute in the genetic susceptibility of individuals to a variety of allergic diseases, because it controls antigen presentation by antigen-presenting cells to helper T cells (3, 4). The results from several studies that have investigated the monogenetic association between mites (*D. pteronyssinus* and *D. farinae*), allergic bronchial asthma and HLA-A,-B,-DR and -DQ antigens and haplotypes are inconsistent (5–8). Several studies of immunogenetic investigation in atopic diseases and allergic bronchial asthma have taken place in several races and nationalities (5, 7, 9). Similar kinds of studies are lacking in Greece, and this is the first familial study that has taken place in our country in children with allergic bronchial asthma.

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Sixty unrelated children, 40 males and 20 females of Hellenic origin and nationality with mite allergic bronchial asthma and all the members of their families (total 263 individuals) were included in the study group. Asthmatic children were recruited from the Allergological Department of Paediatrics, Hippokration Hospital of Thessaloniki. The families were divided in 33 extended and 27 nuclear families. The mean average age of the patients was 9.18 ± 3.64 years. Asthma diagnosis was confirmed according to NHLBI EPR-2 Guidelines' classification (10). The patients included in this study had positive skin prick tests to *D. pteronyssinus* and *D. farinae* (Rast scores > 3.5 U/l) and high levels of total serum IgE. The control subjects consisted of 125 healthy unrelated Greek children without a medical history of atopy. They were healthy children of patients who typed for kidney or marrow transplantation in our laboratory, and they were also typed for HLA-A, -B, -DR and -DQ in order to define HLA haplotypes. The mean age of the controls was 13.28 ± 6.55 years (81 males and 44 females). Age difference between the cases and controls is not statistically significant.

Total IgE was detected with Imx method (Microplate Enzyme Immunoassay, Abott Laboratories). Skin tests were performed using the prick method on 60 asthmatic children. We used the following allergens extracts (ALK, Reagents, UK), *D. pteronyssinus*, *D. farinae*, Cat Epithelia, Dog Epithelia, Grass Pollen Mix and Moulds (*Alternaria alternata*, *Cladosporium herbarum*). The specific IgE antibodies were detected with an enzyme-immunoassay method (Alastat, Diagnostic Products Corporation) using the same antigens as for the prick tests. Results were scored from 0 to IV. An IgE concentration exceeding 0.35 U/l was considered as specific sensitization. Sensitization of other members and controls was verified by Alastat tests using the same procedure and the same panel of allergens throughout the study. Patients and all members of the families were typed for HLA class I and class II antigens. HLA-A and -B typing were performed with the standard microlympho-cytotoxicity (NIH) assay, and HLA-DRB1, DQA1 and DQB1 typing was performed using the polymerase chain reaction with sequence-specific primers (PCR-SSP) low-resolution method, and the HLA-DRB1*04 subtypes have been determined using PCR-SSP high-resolution method. A two-stage complement-dependent microlymphocytotoxicity assay was used to type HLA A and B, antigens (11). T cells were separated from 10 ml fresh heparinized blood by using lymphokwik (One Lamda, Canoga Park, CA), a T-cell separation reagent. The set of sera used in plates Terasaki was provided by the companies Biotest (Dreieich, Germany), Pel Freeze (Wisconsin, WI), One Lamda and Gen Trac (Liberty, NC). Genomic DNA was extracted from 10 ml peripheral blood with 0.1% ethylenediaminetetraacetic acid (EDTA) anticoagulant by the salting-out method as described in the literature (12). A low-resolution analysis of HLA-DRB1* alleles was performed by PCR-SSP

commercially provided [Collaborative Transplant Study (CTS)]. HLA-DRB1*04 subtypes were typed by PCR-SSP (CTS). HLA-DQB1* and HLA-DQA1* typing were performed by PCR-SSP. The nomenclature of PCR-SSP typing is based on the HLA Nomenclature Committee of twelfth International Histocompatibility Workshop and Conference.

The average value of IgE in asthmatic children and the other members of their families was done with $\bar{x} \pm SD$, and the comparison of these values was performed with student's *t*-test. In order to evaluate an HLA association, antigen frequencies were compared with those of the control group using the χ^2 test (epistat 3.0 program). The *P*-values were corrected by multiplying them by the number of antigens tested *P* corrective (*P*_c). When conditions for the χ^2 tests were not suitable (number of antigens below 5), Fisher's exact test was used. *P*_c were considered significant when *P*_c < 0.05 . Relative risks were calculated where a particular genotype showed a significantly increased incidence of mite allergic subjects compared with unrelated controls. Linkage disequilibrium (LD) between all MHC loci was calculated by GOLD (13) program. Qualitative, quantitative and haplotype transmission disequilibrium tests (TDTs) were performed with ASPEX (14), QTDT (15) and TRANSMIT (16) programs, respectively. Alleles with overall lower than 5% were grouped for quantitative and haplotype TDT analyses. Statistical significance was defined at the 5% level.

The average value of IgE in 60 children was $\bar{x} \pm SD = 418.05 \pm 407.46$ IU/ml. The comparison of this value to IgE value of the members of the extended and nuclear families showed a significant difference (*P* < 0.05). The study of the families showed that in 51.70% (31/60) families, one or two parents had detectable specific IgE antibodies in their serum, 94.26% (50/54) mothers and 63.64% (27/54) fathers). In 63.64% (21/33) extended families, we have found one or more specific IgE antibodies in grandfathers and grandmothers (60.6%), 63.63% in grandparents of their mothers and 36.36% in grandparents of their fathers (*P* = 0.04).

MHC class I and II gene analysis after *P*_c showed a significant increase of HLA-DRB1*04 and HLA-DQA1*0301 (Table 1). HLA-DRB1*04 subtypes were not significantly different. TDT revealed that no specific A, B, DRB1, DQA1 or DQB1 allele was transmitted preferentially to affected children. Linkage disequilibrium was assessed by calculation of Lewontin's *D'* (LeD) value for all pairs of loci. There was a significant LD among DRB1*, DQA1* and DQB1* loci only (*D'*: 0.771–0.912), and thus haplotype TDT was restricted to class II MHC antigens. No specific MHC II haplotype was preferentially transmitted to affected children. Quantitative TDT revealed that DQB1* was associated with logarithmically-transformed values of total serum IgE (Table 2). Detailed analysis showed that the susceptible alleles were DQB1*0301–4 (*P* = 0.0006). The study of the HLA haplotypes failed to demonstrate any significant association between any haplotype, maternal or paternal, and mite allergic bronchial asthma in Greek children.

Significant increase ($P < 0.05$) of major histocompatibility complex (MHC) class II allele frequencies in children with allergic bronchial asthma

MHC alleles	%	<i>P</i>	<i>P</i> correction	Odds ratio	Confidence interval	Relative risk
DRB1*04	12.67	0.003	0.4	2.89	1.39–5.99	2.86
DQA1*0301	21.75	0.0009	0.009	3.25	1.57–6.77	3.22

Table 1**Qualitative and quantitative transmission disequilibrium test results**

Locus	Qualitative	Quantitative
A	0.66	0.70
B	0.53	0.15
DRB1	0.19	0.09
DQA1	0.51	0.11
DQB1	0.70	0.06

Table 2

This is the first study of HLA antigens and haplotypes in Hellenic children with bronchial asthma and sensitivity only to mites. Familial and epidemiological studies demonstrated that the specific IgE response to allergens has a relationship to HLA class II genotype. Blumenthal et al. (7) in USA had shown Ra5 ragweed sensitivity linked to different HLA haplotypes in different families, and that in some families, no linkage with HLA was present. The study of Holloway et al. (8) in atopic families in UK, with sensitization to HDM, did not demonstrate any relationship between HLA-DR, -DQ and -DP genotype and atopy. Also, the study of Howell et al. (17) did not demonstrate the relationship between a specific response to allergens of the HDM and HLA class II alleles. Studies by Aron et al. (5) in France found the presence of a low frequency of HLA-DRB1*04 alleles in atopic asthmatic people in comparison to non-atopic. Young et al. (18) in the UK did not find any positive or negative correlation between HLA-DR and -DP antigens and specific response to mites. The significant increase of HLA-DQA1*0301

allele in children in our study which presents positive LD with HLA-DRB1*04 was found in studies by Aron et al. (5) and Holloway et al. (8) in people with increased levels of IgE. The study of O'Brien et al. (19) in the UK, which was done on people with specific sensitivity to *D. pteronyssinus*, showed a negative relationship with HLA-DQ2 and a positive relationship with HLA-DQ7. These results are in agreement with our results for the HLA-DQ2. Analysis of DRB1*04 subtypes did not demonstrate significant difference to any subtype. In the literature, there is no analysis of HLA subtypes of antigens with a significant increase in allergic bronchial asthmatic patients. Analysis of haplotypes did not demonstrate any extended or natural selection haplotype correlated with the appearance of the disease. The study by Lara-Marquez et al. (9) has identified an association of DRB1*1101DQA1*0501DQB1*0301 haplotypes with Dermatophagoides-sensitive atopic asthma in a sample of the Venezuelan population.

In summary, our results suggest that HLA-DRB1*04 and HLA-DQA1*0301 are possibly important factors in the development of atopic asthma in Greek children with sensitivity to mites. No specific MHC II haplotype was preferentially transmitted to affected children. Additionally, we found DQB1*0301–4 as susceptible alleles for the increased value of serum IgE in children with allergic bronchial asthma to mites. Studies should implicate other candidate genes that in combination with HLA predict individuals at risk for the development of mite atopic asthma.

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