

Frequencies of HLA-DR7 and HLA-DR4 Alleles in Hungarian Asthmatic Children with Mite Allergy

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ABSTRACT Certain combinations of alleles within HLA-DRB1,-DQA1,-DQB1 region are associated with susceptibility to allergens causing a well-defined disease as bronchial asthma. Our purpose was to study the HLA-DRB1,-DQA1,-DQB1 allelic polymorphisms in 57 patients with bronchial asthma (unrelated children 4 to 17 years of age) caused by mite allergy and 57 unrelated non-atopic controls and 45 non-mite sensitive asthmatic children (aged 4 to 17 years). We examined the allelic polymorphisms by molecular genetic methods. We found an increased frequency ($P < 0.001$) in the haplotype of HLA-DR7 - DQA1*0201 - DQB1*0202 among patients compared to non-atopic controls and non-mite sensitive asthmatic controls and a decreased frequency ($P \leq 0.01$) of HLA-DR4 - DQA1*0301 - DQB1*0302 haplotype among patients compared to non-atopic controls. Our data suggest that HLA-DR7 seems to be predictive factor to bronchial asthma with mite-sensitive Hungarian children. We found that the haplotype of HLA-DR4 - DQA1*0301 - DQB1*0302 may be significantly implicated in the protection to the disease. We could not find any date in the literature referring to the HLA-DR7 and DR4 alleles associated with mite allergic in European population.

INTRODUCTION

Bronchial asthma can be characterized as a complex, multifactorial disease in which both the genetic microheterogeneity and environmental factors are involved (Barnes et al. 1998). A large number of genes, as many as 12-20 ones, in which some molecular variants are needed to expression of the heterogeneous phenotypes of asthma (Rammensee et al. 1995). The specificity of the immune response to allergens is governed by the trimolecular [Human Leukocyte Antigen (HLA) - peptide - T-cell receptor (TCR)] interaction (Rosenwasser 1998). The highly polymorphic HLA and TCR genes have genetic

determinants of antigen-specific IgE responses (Moffat et al. 1997). In the literature the gene frequency of susceptible alleles shows significant differences in several populations. A positive association between HLA-DR1, DR2, DR3, DR4, DR5, DR6 and DR52 molecules and mite allergy could be demonstrated in geographically separated populations (Okano et al. 1996; O'Hehir et al. 1990; Young et al. 1994; Gao et al. 1998). Until now, two Korean studies have reported, that the allelic frequency of HLA-DRB1*07 was higher and the DRB1*04 was lower in citrus red mite (CRM)-sensitive asthmatics (Cho et al. 2000; Kim et al. 2001). CRMs belongs to the family of spider mites. Bruches et al. (1996) reported that all patients sensitized to spider mites were cross-reactive to house dust mites. The closer the taxonomic relationship between mites, the greater the level of cross-allergenicity (Arlan et al. 1984). The primary aim of the present study was to examine the frequency of HLA-DRB1,-DQA1,-DQB1 alleles in Hungarian asthmatic children caused by mite allergy.

MATERIALS AND METHODS

On the basis of clinical history of asthma, lung function test, skin prick test and the total and specific IgE level in serum 57 unrelated Hungarian children (Group 1: 18 girls and 39 boys, aged 4 to 17 years) complained of asthmatic symptoms corresponding with moderate persistent bronchial asthma by GINA classification of asthma severity (1998) were selected into the examined group. Two control groups were studied, 45 non-mite sensitive asthmatic unrelated children (Group 2: 13 girls and 32 boys, aged 4 to 17 years) with detectable skin-prick positive test and increased specific IgE level to ragweed pollen and 57 unrelated non-atopic healthy adult blood donors (Group 3) with no personal history of asthma or atopy and skin-prick negative test.

In the group 1 all the subjects presented positive immediate hypersensitivity skin test to Dermatophagoides pteronyssinus and Dermato-

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phagoides farinae extracts and in the group 3 positive immediate hypersensitivity skin test to ragweed pollen (Allergopharma, Germany) on the volar surface of the forearm. All the patients had no positivity to any other allergens. The reaction was read at 15 minutes. The diameter of the wheal was required to be ≥ 5 mm and the ratio (wheal to allergen / wheal to histamine: 1 mg/ml) ≥ 0.5 . The negative control was absolutely negative in all cases.

Total IgE levels in serum were determined by using a double antibody radioimmunoassay (Pharmacia IgE RIA, Uppsala, Sweden). Measurement of specific IgE in serum based on chemiluminescence against d1 (Dermatophagoides pteronyssinus, Der p 1), d2 (Dermatophagoides farinae, Der f 1) and W1 (ragweed) was used (Magiclite, CIBA Corning Diagnostics, Denmark).

Genotypes were determined by molecular genetic methods. DNA was extracted from white blood cells of subjects (Woodhead et al. 1986). This procedure was followed by the polymerase chain reaction (PCR) amplification of the second exons of the HLA-DRB1, -DQA1, -DQB1 genes. Genotyping of HLA-DRB1 alleles was carried out with the Dynal RELI SSO HLA-DRB, a direct DNA probe test, after the nucleic acid amplification, uses a nucleic acid hybridization method for the differentiation of 70 HLA-DRB alleles as allele clusters and 9 supertypes (Dynal, Oslo, Norway). We used the terminology DR1-10 because of having taken no account of subtypes in DR region. DQB1 genotypes were identified with the INNO-LiPA DQB PCR-reverse hybridization kit, allowing the discrimination of 30 alleles (Innogenetics, Ghent, Belgium). For DQA1 genotyping we used Ota's PCR restriction

fragment length polymorphism (RFLP) method, differentiating 8 alleles (Ota et al. 1991).

To evaluate differences in alleles comparing our data with that of the controls (group 2 and group 3) we applied the chi square test with a 2 by 2 contingency table using the Yates correction. The significant P values is less than 0.05. Odds ratio (OR) values were calculated.

RESULTS

Clinical parameters are summarized on Table 1. The serum level of total IgE was higher than 100 kU/l in all mite sensitive asthmatics with mean value of 367 kU/l and ranging from 120 to 1491 kU/l and in non-mite sensitive asthmatics with mean value of 360 kU/l and ranging from 94 to 4000 kU/l. Specific IgE antibodies against Der p 1, Der f 1 and W1 were found in both groups of patients. The mean serum level of specific IgE against Der p 1 was 77.7 Relative Like Unit (RLU), against Der f 1 was 48 RLU and against W 1 was 43 RLU.

The frequencies of the HLA class antigens DR and DQ were analyzed comparing the results obtained in mite sensitive asthmatic versus non-atopic groups and in non-mite sensitive versus mite sensitive asthmatic groups. Results are seen on Table 2. No deviation from the Hardy-Weinberg equilibrium has been observed. HLA-DR7-DQA1*0201-DQB1*0202 ($P < 0.001$) haplotype proved to be the most frequent one in comparison with the non-atopic group. There was a statistically significant negative association with the haplotype of HLA-DR4-DQA1*0301-DQB1*0302 ($P \leq 0.01$) in mite sensitive asthmatic versus non-atopic group. Statistically significant differences were remained in the presence of the

Table 1: Clinical parameters

<i>Group 1</i>	<i>Group 2 (mite sensitive asthmatics, n=57)</i>	<i>Group 3 (non-mite sensitive asthmatics, n=45)</i>	<i>(non-atopic controls, n=57)</i>
Girls (%)	31.6	28.9	38.6
Boys (%)	68.4	71.1	61.4
Mean total IgE[kU/l]	367 (120-1491)	360 (94-4000)	-
d1 IgE[RLU]	77.7 (2.6-478)	-	-
d2 IgE[RLU]	4.8 (1.8-298)	-	-
W1 IgE[RLU]	-	43 (11-104)	-

RLU = Relative Like Unit

Table2: Frequencies of HLA-DRB1,-DQA1,-DQB1 alleles: comparison among groups

	Group 1 (mite sensitive asthmatics, n=57)	Group 3 (non-atopic controls, n=57)	P	OR	Group 1 (mite sensitive asthma- tics, n=57)	Group 2 (non- mite sensitive asthmatics, n=45)	P	OR
	No. of alleles				No. of alleles			
DR7	30(53)	7(12)	<0.001	5.46	30(53)	7(16)	0.001	0.23
DR4	4(7)	17(30)	<0.01	0.21	4(7)	8(18)	NS	-
DQA1*0201	31(54)	9(16)	<0.001	4.36	31(54)	9(20)	<0.01	0.29
*0301	5(9)	18(32)	<0.01	0.24	5(9)	9(20)	NS	-
DQB1*0202	26(46)	6(11)	<0.001	5.32	26(40)	7(16)	0.01	0.29
*0302	4(7)	14(25)	=0.01	0.26	4(7)	2(4)	NS	-

Figures in parentheses are percentages.
NS=nonsignificant

HLA-DR7 - DQA1*0201 - DQB1*0202 (P<0.01) haplotype when mite sensitive asthmatics were compared with non-mite sensitive asthmatics. The previously mentioned haplotype was the most prevalent one in the mite sensitive asthmatic group.

DISCUSSION

The fourth less common allele in Hungarians is HLA-DR7 (Varga et al. 1996), while patients with asthma caused by mite allergen carry this allele in high frequency. Studies on *Der p* I showed that T cells recognize the next short peptides of mite allergen: *Der p*I p45-67 (DR7), p117-143 (DR7) (Yssel et al. 1992). It indicates that the antigen-binding sites of T cell epitopes restricted by this HLA-DR7 allele and may be clinically important for asthmatic patients. Concerning HLA-DR7 allele positive association with mite atopy and bronchial asthma could be also found in French patients (Aron et al. 1996), and in the CRM-induced Korean asthmatics (Cho et al. 2000; Kim et al. 2001) whereas a significant decrease of HLA-DR7 allele was detected in the asthmatic children caused by mite allergens in Hellenic population (Parapanissiou et al. 1996). DR7 allele is probably implicated in the susceptibility to the atopic asthma (Senechal et al. 1999). DR7 allele was found significantly increased in the mite sensitive asthmatics studied versus non-mite sensitive asthmatic controls.

HLA-DR4 is the third most frequent allele in Hungarians (Varga et al. 1996), but it is virtually absent corresponding to a 'protective' function in our asthmatic children versus non-atopic controls. Other data could be proven a significant positive association of HLA-DR4 allele in French, Hellenic and Japanese patients with atopic asthma (Aron et al. 1996; Parapanissiou et al.

1996; Okano et al. 1996). A quite strong negative association of HLA-DR4 allele has been demonstrated with mite allergy in Chinese children and in the CRM-induced Korean asthmatics (Kue-Hsiung et al. 1991; Cho et al. 2000; Kim et al. 2001). Our observation referred to the HLA-DR4 allele seems not to be significantly implicated in protection to the disease in the mite sensitive asthmatics studied versus non-mite sensitive asthmatic children.

We can conclude that HLA-DR7 allele as an additional risk factor is significantly involved in the presentation of mite allergens which are related to susceptibility causing the typical phenotype of bronchial asthma, while HLA-DR4 allele may be significantly implicated in the protection to the disease in our patients versus non-atopic controls. We could not find any date referring to these two HLA-DR alleles in European populations.

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