HLA class II in atopic and non atopic members of asthmatic family pedigrees

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ABSTRACT

Asthma is an inflammatory disease with a strong genetic predisposition. There is a strong relationship between the immune response to several antigens, and specific HLA-DQ/DR haplotype. Objectives: The aim of this study was to determine the HLA class II (DRB1 and DQB1) phenotypic frequencies in atopic and non- atopic members of asthmatic family pedigrees and to analyze the relationship between these phenotypes and any type of asthma. Patients and methods: A series of 41 asthmatic individuals from 20 asthmatic family pedigrees assigned either to the atopic (24-AA) or to the non-atopic (17-NAA) groups were studied and compared with 15 healthy age and sex matched controls. HLA class II DRB1 and DQB1 typing were done to all patients and controls using polymerase chain reaction (PCR) technique. Results: The comparison between the AA and NAA groups of patients revealed an increase in DRB1 alleles(01, 103, 15, 16, 17, 18+, 04, 07, 09, 10, 11, 12 ,13.1 ,14.2, 53) and in DQB1 alleles (2,3(8), 3(7), 3(8,9), 2,3(7,9) 4) while DRB1 alleles (08,13.3,13.4,14.1,14.3,14.4,52,51) and DQB1 alleles (5,6,2) were reduced, but none of these tendencies was statistically significant. Also analysis of the AA group and NAA group versus controls showed no statistical significant findings. Conclusion: Although there are some differences in HLA phenotypic distribution in asthma patients, no definitive HLA association could be established with atopic or non atopic asthma in the population studied. Nonetheless, although HLA by itself may not determine the asthma process, this does not exclude its involvement with other genetic systems which may be implicated in the susceptibility to this disease.

INTRODUCTION

Bronchial asthma is a diffuse obstructive lung disease with hyper-reactivity of lower air ways to multiple stimuli, having an episodic character, and a high degree of reversibility whether spontaneously or therapeutically. It is associated with inflammation and has a variable severity[1].

Since a high prevalence of asthma is found in relatives of patients, in twin and interfamilial studies (the risk for a child to have asthma is 25% if one parent has asthma, and about 50% if both parents are affected), it appears that there is a genetic components, as well as environmental factors, which influences its development. However, although research into genetic susceptibility to this pathology has become a very attractive field, because there are many genes that may be implicated, its hereditary transmission has not been clearly established[2,3].

Human leukocyte antigens (HLA) are molecules involved mainly in antigen presentation to T cells. They are encoded by the major histo- compatibility complex (MHC) genes which spans 3.6 million base pairs on the short arm of chromosome number 6. The MHC is organized into 3 classes; I away from centromere, II near to centromere, and III in between I and II. It encodes for classical and non- classical HLA molecules and other molecules having either immunological or non-immunological function[4].

The genes of the HLA system have been identified among candidate genes which may have a role in the patho physiology of asthma[5], but their influence on the development of asthma remains unclear[6]. There are several studies that describe different associations between HLA antigen and asthma in both atopic and non atopic patients[7-9]. However in other studies, no direct association has been found[6,10]. Nonetheless other studies have shown a genetic linkage of asthma susceptibility to the 6 p21.3-23 region, which maps near to the HLA region[11].

The aim of our work was to study the HLA class II (DRB1 and DQB1) phenotypic frequencies in a population of asthmatic family pedigrees and to determine whether there is any antigen associated with any type of asthma.

PATIENTS AND METHODS

A series of 41 asthmatic individuals from 20 asthmatic family pedigrees assigned either to the atopic (24-AA) or to the non-atopic (17-NAA) group were studied and compared with 15 unrelated healthy, age and sex matched donors, with no history of asthma or atopy, as a control group. All cases were selected from Allergy and Immunology Clinic, Children's Hospital, Cairo University according to the definition of the American Thoracic Society[12], on the basis of clinical symptoms (history of wheezing, chest tightness and shortness of breath relieved by an inhaled beta – agonist). Physical examination and pulmonary function tests demonstrated a normal or

reversible airway obstruction manifested by a post- bronchodilator increase in FEV1>15%. Atopic asthmatics had a positive history of a broncho-constrictive response after allergen exposure and one or more positive skin prick test (SPT). Non- atopic asthmatics developed symptoms following respiratory tract infections, but with no history of allergen – induced bronchospasm, no familiar atopy, and negative SPT.

DNA was isolated from 3ml of whole blood using the wizard® Genomic DNA purification kit supplied by Promega. Madison, WL.USA[13]. The primer used in the polymerase chain reaction (PCR) were (G1-A1; H2-A2; H3-A3) for the DRB1 generic amplification, and (H1-A1) for the DQB1. The PCR products were identified using a 2% agarose gel electrophoresis followed by detection of the DNA bands in UV light.

HLA phenotypic frequencies were estimated by direct counting using the (chi)^2 test to make comparisons between patient and control groups. A total of four comparison were made: each group of asthmatic patients versus the control group, atopic patients versus non-atopic ones, and the total group of asthmatic patients versus the control group. The significant level was set at 0.05.

RESULTS

The study subjects consisted of 41 asthmatic individuals from 20 asthmatic family pedigrees; they were divided into two groups; group 1: 24 atopic asthmatics (AA) with mean age of 16 years and group 2: 17 non atopic asthmatics (NAA) with mean age of 12.8 years. Also 15 healthy children with mean age of 13.8 years were included as a control group.

HLA class II (DRB1 and DQB1) phenotypic frequencies were determined in all patients (AA and NAA) and controls. The comparison between the AA and NAA groups of patients revealed an increase in DRB1 alleles (01, 103, 15, 16, 17, 18,+ 04, 07, 09, 10, 11, 12, 13.1, 14.2, 53) and DQB1 alleles (2,3 (8); 3(7); 3 (8, 9), 2,3 (7, 9), 4) while DRB1 alleles (08, 13.3, 13.4, 14.1, 14.3, 14.4, 52, 51) and DQB1 alleles (5, 6, 2) were reduced, but none of these tendencies was statistically significant.

Analysis of the AA group versus control showed an increase in DRB1 alleles [01, 103, 16, 18+, 04, 07, 08, 09, 10, 12, 14.2, 53] and DQB1 alleles [5; 2, 3 (8); 3 (8, 9); 2, 3 (7, 9)], while as DRB1 alleles [15, 17, 11, 13.1, 13.3, 13.4, 14.1, 14.3, 14.4, 52, 51] and DQB1 alleles [6, 2, 3 (7), 4] were decreased, although these differences were not statistically significant.

When the NAA and control group were compared, an increase in the DRB1 alleles [08, 10, 13.3, 14.1, 52] and in the DQB1 alleles [5; 6; 2; 2, 3 (8); 3 (8, 9); 2, 3 (7, 9)] were observed in the patients. By contrast DRB1 alleles [01, 15, 17, 04, 07, 11, 13.1, 13.4, 14.2, 14.3, 14.4, 53, 51] and DQB1 alleles [3 (7); 4] were underrepresented, but not at a level of statistical significance (Tables 1 and 2).

Finally, the asthmatic patients as a whole (AA and NAA) were compared with controls: no statistical significance was found (see Tables 1 and 2).

DISCUSSION

Attempts to identify the association between HLA genes and atopy are currently done taking into consideration the role of HLA molecules in regulating the immune response. Grouping of HLA – DRB1 and DQB1 alleles into functional categories may assist in the search for predictive factors in relation to atopic diseases like bronchial asthma[14].

In this study we report the distribution of HLA class II (DRB1 and DQB1) phenotypes in 20 Egyptian family pedigrees with a clinical diagnosis of asthma. Although the results show heterogeneity, there is evidence of differences in phenotypic frequencies between patients and healthy controls, and between the two groups of asthmatic patients (AA and NAA).

In the class II analysis we observed that patients bearing some alleles of the HLA-DRB1 developed atopic asthma more frequently, which is in agreement with the described associations between DR3 and the IgE response to some common allergens, such as those from house dust mite and rye grass[15,16]. Other authors reported that DR4 and DR7 genotypes are positively associated with atopic asthma[17]. This association was not found in our series, but the population, the selection, and the numbers of patients and controls were different. In fact, when atopic (SPT – Positive) and non atopic (SPT – negative) asthmatics were compared, we found that HLA – DRB1 alleles (01, 103, 15, 16, 17, 18+, 04, 07, 09, 10, 11, 12, 13.1, 14.2, 53) were more frequent in atopic patient, although this findings were not statistically significant.

With regard to HLA – DQB1, studies of childhood asthma suggested a linkage between HLA – DQw2 and atopic asthma[8,18]. In our study, the HLA – DQB1 alleles[5,6,2] genotypes increased in non- atopic asthma, but not statistically significant which are in agreement with the results of Torio et al.,[6].

Interestingly, there is evidence that the HLA – DQB1 (0302) genotypes may determine susceptibility to certain autoimmune diseases, such as diabetes[19,20]. On the other hand, taking into account the suggested involvement of certain viruses in the development of asthma [21,22], it is tempting to investigate the role of HLA – DQ restriction for viral or other mimetic peptides that may be implicated in non – atopic asthma, such as occurs in other immune – pathological process[23].

In conclusion, although there are some differences in HLA Phenotypic distribution in asthma patients, no definitive HLA association could be established with atopic or non – atopic asthma in the patients studied. Nonetheless, although HLA by itself may not determine the asthma process, this does not exclude the involvement with other genetic systems which may be implicated in the susceptibility to this disease.

Table 1
HLA – DRB1 genotype frequencies in the three studied groups

Allele DRB1	Genotype frequency No. (%)			P
	Atopic (N=24)	Non atopic (n=17)	Control (n=15)	
01	3 (12.5)	1 (5.9)	1 (6.7)	ns
103	1 (4.2)	0 (0)	0 (0)	ns
15	5 (20.8)	1 (5.9)	4 (26.7)	ns
16	2 (8.3)	0 (0)	0 (0)	ns
17	3 (12.5)	2 (11.8)	2 (13.3)	ns
18+	1 (4.2)	0 (0)	0 (0)	ns
04	5 (20.8)	3 (17.6)	3 (20.0)	ns
07	5 (20.8)	2 (11.8)	3 (20.0)	ns
08	1 (4.2)	1 (5.9)	0 (0)	ns
09	2 (8.3)	0 (0)	0 (0)	ns
10	3 (12.5)	1 (5.9)	0 (0)	ns
11	3 (12.5)	2 (11.8)	4 (26.7)	ns
12	4 (16.7)	0 (0)	0 (0)	ns
13.1	4 (16.7)	2 (11.8)	3 (20.0)	ns
13.3	5 (20.8)	5 (29.4)	4 (26.7)	ns
13.4	2 (8.3)	2 (11.8)	2 (13.3)	ns
14.1	2 (8.3)	3 (17.6)	2 (13.3)	ns
14.2	2 (8.3)	1 (5.9)	1 (6.7)	ns
14.3	0 (0)	1 (5.9)	1 (6.7)	ns
14.4	2 (8.3)	2 (11.8)	2 (13.3)	ns
52	7 (29.2)	7 (41.2)	5 (33.3)	ns
53	9 (37.5)	4 (23.5)	4 (26.7)	ns
51	2 (8.3)	3 (17.6)	3 (20.0)	ns

ns = P >0.05 both Atopic vs Non atopic, Atopic vs Control, Non atopic vs control

Table 2
HLA – DRB1 genotype frequencies in the three studied groups

Allele DRB1	Genotype frequency No. (%)			P
	Atopic (N=24)	Non atopic (n=17)	Control (n=15)	
5	4 (16.7)	4 (23.5)	2 (13.3)	ns
6	5 (20.8)	6 (35.3)	4 (26.7)	ns
2	4 (16.7)	5 (29.4)	3 (20.0)	ns
2, 3 (8)	5 (20.8)	3 (17.6)	2 (13.3)	ns
3 (7)	1 (4.2)	0 (0)	2 (13.3)	ns
3 (8, 9)	4 (16.7)	2 (11.8)	1 (6.7)	ns
2, 3 (7, 9)	5 (20.8)	3 (17.6)	2 (13.3)	ns
4	3 (12.5)	2 (11.8)	3 (20.0)	ns

ns = P >0.05 both Atopic vs Non atopic, Atopic vs Control, Non atopic vs control

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