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Association of HLA class II Alleles with Childhood Asthma and Total IgE Levels

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ABSTRACT

Asthma is a complex and multifactorial disorder. Several studies have reported association between different HLA- DQB1 and HLA- DRB1 alleles and allergic asthma. The aim of the present study was to investigate the association of HLA-class II alleles and haplotypes, with total serum IgE and the results of the skin prick test in Iranian children with allergic asthma.

A total of 112 patients with allergic asthma symptoms (75 males and 37 females) were selected randomly from the pediatric hospital. In some patients total serum IgE and prick test were determined.

Data of this study shows that HLA-DRB1*12 significantly increased in asthmatic patients (4.5% *vs.* 0%, P-value=0.04). HLA-DQB1*0603 and 0604 alleles were significantly higher in asthmatics than those in normal controls (10% *vs.* 0%, P-value= 0.0001; and 9.3% *vs.* 3.7%, P-value= 0.04, respectively). The statistical significance was relinquished after *p* value correction for all alleles except for HLA-DQB1*0602 (P_c=0.03) and HLA-DQB1*0603 (P_c=0.0015). Conversely, HLA-DQB1*0501 and 0602 were decreased in asthmatics compared to normal controls (7.5% *vs.* 13.5%, P-value= 0.05; and 4% *vs.* 12.5%, P-value= 0.002, respectively). The mean of total IgE in patients was 483 IU, and it was significantly high about 1140 IU in asthmatic patients with positive skin prick test to house dust. The most frequent alleles in asthmatic patients with the total IgE>200 IU/mL were HLA-DRB1*11and 1401, HLA-DQA1*0505, HLA-DQB1*0301 and in patients with total IgE<200 IU/mL were HLA-DRB1*0301, 07 and 1301, HLADQA1*0201 and 0301, HLA-DQB1*0201.

These data suggests that HLA-DRB1, DQA1 & DQB1 alleles and haplotypes might be implicated in susceptibility to allergy and asthma and serum IgE production. As asthma and atopy are multifactorial disorders, probably HLA genes are involved in the regulation of immune specific responses to common allergen.

Key words: Asthma; HLA antigens; IgE

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INTRODUCTION

Asthma is an inflammatory disease of the airway, characterized by airway hyperreactivity, chronic eosi-

nophilic inflammation, episodes of reversible bronchoconstriction, and mucus hypersecretion.

The world wide prevalence of asthma is increasing within the last 30 years with the highest frequency in Wales, Australia, New Zealand and North-west European countries.[1]

Recent reports in Iran showed the asthma prevalence of 6-9% in adolescent students.[2] Gene with environment interaction is an important component in the eventual expression of asthma.[3] Genome-wide screening studies have identified multiple chromosomal regions containing susceptibility genes for asthma such as 2q, 3p, 5q, 6p21 and 12q23.[4] A first step in the development of allergic diseases and inflammation is sensitization to allergen and production of IgE, showing the important role of Th2 cytokines in asthma.[5] It has been reported that there is an association between serum IgE level and asthma development.[9]

Total IgE level is mainly determined by genetic factors while other factors such as environmental exposures are less important determinants of IgE level.[10,11] Several chromosomal linkage such as 5q and 11q13 have been identified to affect serum IgE level.[12,13] Furthermore, IgE production in patients with allergic asthma has been suggested to be associated with certain HLA class II alleles.[14] The human leukocyte antigen (HLA) complex located on chromosome 6p21, may play an important role in the genetic basis of childhood asthma.[6]

HLA class II antigens have been shown to restrict the antigen presentation of specific allergen peptides through antigen presenting cells (APC) to CD4+ T-cells.

HLA class II restricted IgE production against certain part of allergens explains in part the genetic susceptibility of subjects with allergic asthma.[7]

The aim of the present study was to investigate the HLA class II alleles and haplotypes frequency in a group of pediatric patients with allergic asthma and to compare with those of a normal nonasthmatic group. Furthermore, we have analyzed the possible association of certain HLA alleles with specific allergen sensitivity and the total serum IgE level.

PATIENTS AND METHODS

Subjects

One hundred and twelve unrelated patients with allergic asthma (75 males, 37 females, mean age 6±2), referred to Children Medical Center at Tehran University were enrolled in this study. All of the patients were diagnosed based on the definition of American Thoracic Society including clinical symptoms of asthma (wheezing, chest tightness, dyspnea relieved by an inhalation ß-agonist) NHLBI EPR-2 Guidelines' criteria.[15]

Median value of total serum IgE level in patients with available data was 483 IU/ml.

Skin prick tests (SPT) were performed using common allergens in Iran such as Dust, dander, cockroach, food additives, cigarette, cocoa, weeds, grass, mite, strawberry and tree.

Eighty normal blood donors with no history of atopy, asthma and allergic diseases were randomly selected as control group. Written informed consents were obtained from the parents of children with asthma and control normal blood donors.

HLA Class II Typing

DNA was extracted from 5 ml of venous blood samples of each of the participants using modified salting-out method. HLA DRB1, DQA1 and DQB1 typing was performed by polymerase chain reaction based on sequence specific primers (PCR-SSP), following Olerup and Zetterquist method.[16]

The DRB, DQA and DQB primers were supplied by the Biotest (Heidelberg, Germany). In the standard kit, low resolution SSP, Ampli TAQ DNA polymerase was used from the Roche (Basel, Switzerland).

The PCR reactions were carried out in 10 µl volumes. The samples were amplified in techne genius thermal cycles. Samples were amplified after initial denaturation at 94 Celsius grade for two minutes, followed by 10 cycles of 94 C denaturation for 10 seconds, 65 C annealing and extension for 60 seconds, and finally 20 cycles of 94 C denaturation for 10 seconds, 61 C annealing for 50 seconds and 72 C extension for 30 seconds. After amplification, the PCR product was electrophoresed on an agarose gel at 170 voltage for 15 minutes, and then on an UV trans-illuminator, the gel was interpreted for specific bands.

The haplotypes were calculated according to linkage disequilibrium among DR, DQA, and DQB alleles.

Statistical Analysis

HLA frequencies were determined by direct counting. The frequency of each allele was compared between asthmatics and normal control group using chi-square test fisher exact test. Patients with available data for serum IgE level were divided into two subgroups

according to the serum IgE level of 200 IU/mL (IgE<200 and IgE>200 IU/ml). The distribution of HLA alleles between these two groups was compared. Statistical analysis was performed using InStat version 3.06 (GraphPad Software Inc, CA, 2003). For the multiple analysis performed in each locus, *P*-values were corrected using Bonferroni correction.[17]

RESULTS

Distribution of DRB1, DQA1 and DQB1 Allele frequencies in Asthmatics and Control Group

As shown in table 1, HLA-DRB1*12 allele frequency was significantly higher in patients than in controls (4.5% vs 0%, *P*=0.04). There was no significant difference in DQA1 allele frequencies. The allelic frequency of DQB1*0501 and DQB1*0602 were significantly lower in patients compared to normal controls (7.5% vs. 13.7%, *P*=0.05; 4% vs. 12.5%, *P*=0.002). Conversely, HLA-DQB1*0603 and DQB1*0604 alleles were significantly more frequent in asthmatics than in control group (10% vs. 0%, *P*=0.0001; 9.3% vs. 3.7%, *P*=0.04).

The only significant result for haplotype association with asthma was DRB1*12/DQA1*0505/DQB1*0301 (4.5% vs. 0.6%, *P*=0.02) (data not shown). The statistical significance was relinquished after *p* value correction for all alleles except for HLA-DQB1*0602 (P_c=0.03) and HLA-DQB1*0603 (P_c=0.0015).

Table 1. HLA class II allele frequencies in Iranian patients with Asthma and control.

HLA Class II alleles	Allergic Asthma n=112 (%)	Control n=80 (%)	Odds ratio (95% CI)	P-value
DRB1-0101	12(5.4)	13(8.1)	0.67(0.3-1.53)	0.4
DRB1-15	28(12.5)	23(14.3)	0.85(0.47-1.54)	0.64
DRB1-16	6(2.7)	3(1.8)	1.44(0.35-5.8)	0.73
DRB1-0301	20(9)	12(7.5)	1.2(0.57-2.50)	0.69
DRB1-04	18(8)	12(7.5)	1.07(0.5-2.3)	1
DRB1-07	28(12.5)	23(14.3)	0.82(0.43-1.57)	0.62
DRB1-08	10(4.5)	5(3.1)	1.44(0.48-4.30)	0.59
DRB1-1001	5(2.2)	4(2.5)	0.89(0.23-3.3)	1
DRB1-11	40(17.5)	39(24.3)	0.57(0.32-1.04)	0.07
DRB1-12	10(4.5)	0(0)	16.71(0.91-285.32)	0.005
DRB1-13	35(15.5)	18(11.2)	1.56 (0.79-3.02)	0.18
DRB1-1401	12(5.3)	7(4.37)	1.3 (0.52 -3.4)	0.8
DQA1-0101	15(6.7)	13(8.1)	0.81 (0.37 – 1.75)	0.69
DQA1-01021	38(17)	20(12.5)	1.4(0.79- 2.5)	0.25
DQA1-0103	37(16.5)	22(13.7)	1.24(0.7-2.19)	0.47
DQA1-0104	10(4.5)	15(9.3)	0.45(0.19-1.08)	0.06
DQA1-0201	23(10.2)	21(13.1)	0.75(0.4-1.42)	0.4
DQA1-03011	27(12.1)	12(7.5)	1.69(0.82-3.4)	0.17
DQA1-0401	8(3.5)	5(3.1)	1.14(0.36 – 3.57)	1
DQA1-0501	20(8.9)	15(9.3)	0.94(0.46-1.91)	1
DQA1-0505	46(20.5)	37(23.1)	0.85(0.52 – 1.4)	0.6
DQB1-0201	41(18.3)	30(18.7)	0.97(0.57 – 1.63)	1
DQB1-03011	50(22.5)	37(23.1)	0.95 (0.58 – 1.55)	0.9
DQB1-0302	20(10.2)	7(4.4)	2.14 (0.88 – 5.1)	0.1
DQB1-0401	7(3.1)	4(2.5)	1.25 (0.36 – 4.3)	0.71
DQB1-0501	17(7.5)	22(13.7)	0.51(0.26-1.09)	0.05[b]
DQB1-05031	11(5)	9(5.6)	0.86(0.35 – 2.1)	0.8
DQB1-06011	16(7.1)	11(6.9)	1.04 (0.47 – 2.31)	1
DQB1-0602	9(4)	20(12.5)	0.29(0.12 - 0.66)	0.002[c]
DQB1-0603	22(10)	0(0)	35.6(2.14 – 592.88)	<0.0001[d]
DQB1-0604	21(9.3)	6(3.7)	2.65(1.04 – 6.7)	0.04[e]

[a,b,e] *P*<0.05, *Pc*= not significant, [c]*Pc*=0.03, d*Pc*= 0.0015

Distribution of DRB1, DQA1 and DQB1 Allele frequencies among asthmatics with IgE<200 and IgE>200 IU/ml

As shown in Table 2, the HLA-DRB1*0301 and DRB1*0701 alleles were more frequent in asthmatic with low IgE level production (17.5% vs. 3.3%, P_c=0.013; 20% vs. 3.3%, P_c=0.0026), while DRB1*1101 was significantly associated with high level of IgE production (40% vs. 7.5%, P_c=0.0013). Furthermore, HLA-DQB1*0301 and DQA1*0505 were associated with high level of IgE production (36.7% vs. 6.7%, P_c=0.0013) and HLA-DQA1*0201,0301 and HLA-DQB1*0201 with low level of IgE production.

Skin Prick Test

Skin prick test was negative in 19.6% of asthmatics with the median IgE level of 296.2 IU/ml. Highest IgE level production observed in those patients with positive SPT result for Dust (11%) (Median IgE level: 1140 IU/ml). Positive SPT results for trees, mites and weeds were similarly distributed and associated with similar IgE level (380, 342 and 478 IU/ml, respectively), while the median IgE level in asthmatics was 483 IU/ml. There was no significant association in HLA alleles and positive skin prick test.

DISCUSSION

There are several genome-wide studies showing the role of genetic factors such as those located in 2q, 3p, 5q, 6p21 and 12q23 chromosomal regions.[4]

The results of the present study, indicate that several HLA class II alleles located on chromosome 6p21, may serve as susceptibility or protective genes against asthma in children. HLA class II alleles and haplotypes analysis in this study showed that DRB1*12, DQB1*0603 and DQB1*0604 alleles might confer one of the genetic predisposing factor for asthma (P=0.04, P=0.0001 and P=0.04, respectively).

Table 2. HLA class II allele frequencies in asthmatic patients and total serum IgE levels

HLA Class II alleles	Allergic Asthma IgE > 200IU	Allergic Asthma IgE < 200IU	Odds ratio (Cl 95%)	P-value
DRB1-0101	6.7%	5%	1.4(0.4-4.6)	1
DRB1-15	13.3%	12.5%	1.09(0.43-2.57)	1
DRB1-0301	3.3%	17.5%	0.15(0.04-0.53)	0.001[a]
DRB1-04	3.3%	10%	0.27(0.7-1.3)	0.08
DRB1-07	3.3%	20%	0.12(0.03-0.4)	0.0002[b]
DRB1-11	40%	7.5%	8.8(3.7-21)	0.0001[c]
DRB1-13	6.6%	20%	0.25 (0.09-0.6)	0.005[f]
DRB1-1401	10%	2.5%	5.4 (1.1 -5.4)	0.03[g]
DQA1-0101	6.7%	7.1%	0.9 (0.3 – 2.75)	1
DQA1-01021	10%	19.1%	0.4(0.2- 1.07)	0.1
DQA1-0103	16.5%	19.1%	0.8(0.4-1.7)	0.7
DQA1-0201	6.7%	19.1%	0.2(0.1-0.7)	0.009[h]
DQA1-03011	3.3%	14.3%	0.19(0.05-0.68)	0.009[i]
DQA1-0501	3.3%	9.5%	0.31(0.08-1.08)	0.13
DQA1-0505	36.7%	6.7%	4.5(2.1 – 9.4)	<0.0001[d]
DQB1-0201	6.7%	19.1%	0.2(0.1-0.7)	0.009[j]
DQB1-03011	36.7%	6.7%	4.5(2.1 – 9.4)	<0.0001[e]
DQB1-0302	3.3%	11.9%	0.2(0.06 – 0.9)	0.04[k]
DQB1-0501	3.3%	9.5%	0.31(0.8-1.1)	0.13
DQB1-06011	6.7%	4.8%	1.5 (0.4 – 5.61)	0.7
DQB1-0602	3.3%	2.4%	1.5(0.24 – 9.2)	1
DQB1-0603	10%	16.7%	0.5(0.25 – 1.3)	0.29
DQB1-0604	6.7%	9.5%	0.6(0.2 – 1.8)	0.59

[a]Pc=0.013, bPc=0.0026, [c]Pc=0.0013, [d,e]Pc=0.0013

[f,g,h,i,j,k] P<0.05, Pc= not significant

Conversely, HLA-DQB1*0501 and DQB1*0602 alleles seem to play as protective genetic factors against asthma development (P=0.05 and P=0.0002, respectively). Haplotypic analysis showed that the only haplotype with significant positive association with asthma was DRB1*12/DQA1*0505/DQB1*0301 (P=0.02).

Following Bonferroni's correction for multiple analysis, the statistical significance was relinquished for all alleles except for HLA-DQB1*0602 (P_c=0.03) and HLA-DQB1*0603 (P_c=0.0015).

In a study by Jinming G *et al.*, HLA-DQA1*0104 and -DQB1*0201 were positively associated with asthma while HLA-DQA1*0301 and -DQB1*0301 alleles were negatively associated with this disorder.[8]

Lara Marquez *et al*., studied the association of HLA alleles with *Dermatophagoides* species sensitive asthmatics in Venezuelan population and identified the role of HLA-DRB1*11 as susceptibility factor while in our population it was somewhat more common in controls that in asthmatics (17.5% vs. 24.3%). This difference may be attributed to some genetic impact on the specific allergen sensitive asthma and to some degree to the genetic population differences.[18]

A Korean study reported the higher frequency of HLA-DRB1*07 in mite sensitive asthmatics than in controls (Pc=0.01).[19]

The study by Parapanissiou E. *et al*., in Greek children with allergic asthma revealed that DRB1*04 and DQA1*0301 might be important factors in susceptibility to asthma with sensitivity to mites.[20]

In the present study, HLA alleles were similarly distributed in patients with sensitivity to certain allergens.

In the second part of this study, we analyzed the association of certain HLA alleles with the low or high level of total IgE production in asthmatics. We found that HLA-DRB1*0301 and DRB1*0701 alleles might be associated with low level of IgE production while DRB1*1101 and 1401, HLA-DQB1*0301 and DQA1*0505 are associated with high total serum IgE level. Our results for DQB1*0301 is inconsistent with the study in Greece.[20] In a study by Kowalski LM. *et al*., there was a strong association between DRB1*01 allele and high total IgE level while DRB1*0701 allele was more frequent in patients with low IgE level.[21]

Several other studies in Germany and UK have indicated that DRB1*0701 might be associated with high IgE production.[22,23]

In conclusion, it is suggested that HLA-DQB1*0603 and DQB1*0602 alleles might be the possible genetic factors for susceptibility or protection against asthma. Furthermore, HLA-DRB1*0301 and DRB1*0701 alleles are associated with low serum IgE level and HLA-DRB1*11, DQB1*0301, DQA1*0505 are associated with high serum IgE in asthmatics

This is the first study showing HLA allele association with asthma in Iranian population. Future studies are needed to investigate the association of HLA alleles in patients with allergic asthma to certain allergens. Also, the newly identified HLA alleles associated with total IgE level should be confirmed through another study using allergen-specific IgE level.

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