Association of HLA-Class II and IgE Serum Levels in Pediatric Asthma

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ABSTRACT

Background: Pediatric bronchial asthma is associated with considerable morbidity. The study was carried out to examine the association of Human Leukocyte Antigen (HLA)-Class II with the disease as we found no similar study on Asian Indian population. **Objective:** To define the HLA-Class II antigens in Asian Indian pediatric patients with asthma. **Methods:** A total of 103 children with asthma and 152 controls were analysed for HLA Class II (DRB1, DQB1and DPB1) by PCR-SSP (Sequence Specific Primers) method. Total serum IgE levels were determined by ELISA assay. **Results:** A positive family history was recorded in 59 patients **(**57%) and 13 (8.5%) of healthy controls. Serum IgE levels were more than normal range in 72% of the patients and 33% of healthy subjects with mean values of 4877 and 627 IU/ml, respectively. DRB1*04 and DQB1*03 showed significant positive relations while DRB1*15 showed a negative association with asthma. DQB1*02 was more common in healthy individuals but was not statistically significant. **Conclusions:** A positive association of the DR4/DQB1*03 and a negative association of DRB1*15 was seen with extrinsic bronchial asthma. However, more studies are required on larger populations to confirm the association of HLA Class II alleles in Indians before a particular allele can be labeled as being protective or causative for asthma.

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INTRODUCTION

Bronchial asthma affects nearly 14 million people worldwide and has been steadily increasing in frequency for the past five decades. Environmental factors clearly influence the onset, progression, and severity of this disease. Family and twin studies indicate that genetic variation also influences susceptibility (1). Atopic asthma is the commonest type of asthma and is a classic example of type 1 associated hypersensitivity reaction which usually commences in childhood (2). It is characterized by airway hyper reactivity, episodes of reversible bronchoconstriction, mucus hypersecretion and eosinophilia. Although many genes have been implicated in the pathogenesis of asthma, this study was limited to studying the association of HLA class II and elevated serum Immunoglobulin E (IgE) with the disease. Sensitization to allergens and IgE production is the first step in development of allergy and inflammation which highlights the role of T_H2 cytokines in asthma (3). Association between serum IgE level and asthma has been reported previously (4-6).

Both genetic and environmental factors have a role in an individual's total serum IgE levels, however, the genetic factors have a greater role (7). It is postulated that inheritance of susceptibility genes leads to the development of a strong T_H2 reaction against environmental allergens. HLA-class II antigens may play an important role in the pathophysiology of allergic inflammation by influencing specific IgE responses. HLA class II antigens have been shown to restrict the antigen presentation of specific allergen peptides by antigen presenting cells to CD4+ T cells which can partly account for the genetic susceptibility of asthma.

MATERIALS AND METHODS

This study included 103 children with bronchial asthma and 152 healthy individuals with no clinical history of asthma. All individuals had been examined in pediatric or medical outpatient department followed by laboratory and pulmonary function evaluation. Patients with asthma were further classified into intermittent, mild, moderate and severe persistent types GINA 2009 criteria (8). All patients and controls were tested for DRB1 and DQB1 and only 45 samples were tested for DPB1 alleles. Due to budgetary constraints not all controls were typed for DPB1, and DQB1 alleles.

Inclusion criteria for asthmatic group:

1) Age less than 14 years,
2) Definite clinical diagnosis of bronchial asthma by pediatrician supported by pulmonary function tests in compliant children,
3) Recurring episodes of non-productive cough, breathlessness and wheezing, having family history in some with reversibility of airway obstruction when treated with bronchodilators alone or with corticosteroids,
4) Cough variant asthma where asthma causes chronic cough. These patients do not wheeze. They have dry hacking cough which is more irritating at night. Cough may occur at anytime of the day. Pulmonary function tests with methacholine challenge test confirm the diagnosis. A therapeutic trial of bronchodilator shows a response in 3 to 5 days,

5) Children under five years were diagnosed on basis asthma predictive index with major and minor criteria and clinically reversible bronchoconstriction which responded to bronchodilators.

Exclusion Criteria. History of Pulmonary Tuberculosis or other restrictive lung diseases, also excluded were children with congenital anomalies including congenital heart diseases. Cystic fibrosis was excluded on the basis of history of recurrent pneumonia, malabsorption and failure to thrive.

Ethical Committee. Clearance had been accorded by the Institutional Ethical committee for carrying out the study. Informed consent was obtained from parents of all individuals and also from control population.

Samples. Whole blood samples were collected in Potassium EDTA and sterile vacutainers for DNA extraction and serum IgE estimation, respectively. DNA was extracted by column based method manually using kits from Qiagen, (Germany) as mentioned previously (9). HLA Class II typing was done by Olerup SSP^{TM} kits (Austria). DRB1 was typed for 103 patients and 152 controls, DQB1 high resolution typing was performed for 49 patients and 39 controls, DPB1 was typed for 45 patients and 48 controls. The post PCR amplified products were examined by loading 10µl in 2% molecular grade agarose gel and visualised with the help of UV Tec Gel documentation system (UK) the Stat score software was used for analysis. Further the analysis was confirmed manually with the help of tables when required. Relevant DRB3/4/5 and DQB1 associations were looked in to for all samples.

Total serum IgE was determined in duplicate by Monobind ELISA kit (Lilac, India). The kit also consists of six human serum references which are used as calibrators from 0-400 IU/ ml. The normal values for IgE were 0-46 IU/ml for up to 3 years – and 0-280 IU/ml for 4-12 years). The intra assay precision was 1.95-5.87% and the inter assay precision for the kit was 3.52-8.42 %.

Statistical Analysis. The frequencies of HLA- class II antigens and alleles in patient and control groups were compared using Epi-info 6 software. Odds ratio (OR) was calculated with 95% confidence intervals (CI). P values were calculated using Chi-square test. Mantel-Haenzel's or Yates correction were applied when needed.

RESULTS

The patients'age ranged from two months to 13 years including 66 boys and 37 girls. CBC was normal in 70 samples; leukocytosis was seen in 23 and eosinophilia in 17 children. Forty-four children (43%) did not have a positive family history. The asthma was severe in 11, moderate in 43 and mild in 49 patients. Frequency of DRB1, DQB1 and DPB1 alleles in patients and controls is shown in Tables 1-3.

Serum IgE levels were normal in 28% of the patients, 71 had higher than expected for their age with 15% having higher than 1000 IU/ml and peak value was 4877 IU/ml. The corresponding values in control population were much lower. Severity of asthma did not show a correlation with total serum IgE levels as it was higher in some patients with mild or moderate asthma than the patients with severe asthma. Thirty-four DPB1 and 17 DQB1 alleles were detected on high resolution typing which reflected considerable heterogeneity in the population studied.

Table 1. Frequency of DRB1 and DQB1 antigens in patients with asthma compared to healthy controls.

DRB1/DQB1 Alleles	Patients n = 103	Controls n = 152	OR (CI 95%)	P Value
DRB1*01	7	12	0.85 (0.34-2.24)	0.95
DRB1*15	35	80	0.46 (0.28-0.78)	0.003
DRB1*16	2	6	0.48 (0.10-2.44)	0.367
DRB1*03	16	20	1.21 (0.60-2.47)	0.593
DRB1*04	26	13	3.61 (1.75-7.43)	< 0.001
DRB1*07	36	41	1.45 (0.85-2.50)	0.173
DRB1*08	6	8	1.11 (0.37-3.31)	0.847
DRB1*09	2	2	1.49 (0.21-10.72)	0.693
DRB1*10	15	22	1.01 (0.50-2.05)	0.951
DRB1*11	19	20	1.49 (0.75-2.96)	0.250
DRB1*12	5	12	0.60 (0.20-1.74)	0.340
DRB1*13	18	32	0.81 (0.43-1.53)	0.480
DRB1*14	14	15	1.44 (0.66-3.12)	0.358
DQB1*02	42	22	0.91 (0.46-1.79)	0.780
DQB1*03	41	30	0.46 (0.23 -0.92)	0.026
DQB1*04	05	1	2.55 (0.29 -22.43)	0.382
DQB1*05	40	22	0.84 (0.42-1.65)	0.608
DQB1*06	51	25	1.02 (0.52-2.00)	0.864

Table 2. Frequency of DQB1 alleles in asthma patients with asthma and controls.

Allele	Patients n = 49	Controls n = 39	Odds Ratio (95%CI)	P Value
*02:01	20	9	2.69 (1.08-6.69)	0.031
*02:02	7	8	1.42 (0.53-3.76)	0.483
*02:03	0	1	--	
*03:01	11	9	0.81 (0.30-2.20)	0.686
*03:02	4	4	0.67 (0.16-2.87)	0.591
*03:03	4	3	0.91 (0.19-4.28)	0.920
*03:17	1	4	0.16 (0.02-1.48)	0.069
*03:19	2	0	-	
*05:01	12	11	0.69 (0.27-1.79)	0.448
*05:02	4	3	0.92 (0.19-4.38)	0.920
*05:03	5	4	0.86 (0.2-3.42)	0.828
*06:01	19	11	1.37 (0.54-3.18)	0.555
*06:02	2	2	0.69 (0.09-5.08)	0.710
*06:03	6	8	0.47 (0.15-1.47)	0.185
*06:04	0	1	--	
*06:09	1	0	--	
*06:11	1	0	--	

Table 3. Frequency of DPB1 alleles in patients and controls.

DPB1 Alleles	Patients n =45	Controls n= 48	Odds Ratio (95% CI)	P Value
*01:01	2	4	0.88 (0.09-2.94)	0.81
*02:01	16	16	0.91(0.47-2.60)	0.84
*03:01	2	1	2.44(0.19-2.49)	0.88
*04:01	23	32	1.15 (0.23-1.21)	0.38
*04:02	7	8	0.87 (0.30-2.79)	0.81
*05:02	0	2	-	
*09:01	6	3	2.11 (0.54-9.85)	0.53
*13:01	7	9	1.07 (0.27-2.36)	0.9
*14:01	3	3	1.20 (0.20-5.60)	0.84
*15:01	3	0	-	
*17:01	0	2	-	
*26:01	3	5	0.56 (0.14-2.73)	0.66
*53:01	2	0	-	
*80:01	0	2	-	
*94:01	2	0	-	

DPB1alleles with a frequency of one are not shown in the table but included the following:
*05:01, *06:02, *08:01, *10:01, *11:01, *11:02, *13:02, *16:01, *22:01, *23:01, *23:02, *27:01, *28:02, *33:01, *39:01, *51:01, *77:01, *88:01 and *96:01

DISCUSSION

Asthma runs strongly in families and its heritability has been estimated at 60% (4). Childhood asthma is more common in boys than girls whereas adult onset asthma is more common in women. Our study also had a preponderance of boys and a positive family history was present in 57% of individuals thus the results were consistent to that mentioned in another study and in a genome wide study on asthma (10,11).

Total IgE serum levels were used as a marker of atopy and it was raised in two-third of the children with much higher peak values as compared to controls. DRB1*04, DQB1*03 and DQB1*02:01 alleles showed significant positive association with asthma while DRB1*15 was negatively associated with the disease. The results indicate that some HLA class II alleles may lead to susceptibility or be protective in pediatric asthma. We postulate that DRB1*04 ($p<0.0001$) might confer one of the predisposing factor for asthma. Conversely, DRB1*15 was reported to play a protective role against asthma development in Asian Indian population (12). This allele is the most common class II allele in this population which may also account for the relative lower incidence of asthma as compared to other regions of the world. The higher frequency of DQB1*03 could be due to common DR4/DQ3 association. DQB1*02:01 allele showed a significant positive association with asthma which was associated with DRB1*07 in addition to DRB1*03. On searching "http:// www.allele.fequencies.net/" we found these haplotypes being reported from India although DRB1*07 is commonly associated with DQB1*02:02. There were many patients positive for these DRB1*07 although significant association was not observed.

Parapanissiou *et al.* in two studies done on Greek children with asthma showed that presence of DRB1*04 was an important factor in the development of atopic asthma in

Greek children (13,14). Two Korean studies have shown contradictory findings in which DRB1*07 showed positive association with citrus red mite and house dust mite sensitive asthma and was associated with development of sensitization to *Dermatophagoides pteronyssinus*. However, DRB1*04 showed positive association with former but prevented development of sensitization to *Dermatophagoides pteronyssinus* (15,16). It is therefore likely that in different ethnic groups different HLA antigens have a role in predisposing or preventing the disease.

HLA-DR has been shown to have a significant genome wide association with total serum IgE level but not with asthma in a large recent genome wise study involving 10,365 patients with asthma (10). Woszczek *et al.* found a strong association of DRB1*02 (DRB1*15/*16) with asthma in grass pollen sensitive children (17). A study on Southern Chinese population show no association of asthma or serum IgE levels with DR and DQ antigens, whereas another study showed a positive association of asthma with DRB1*15 antigen which is contradictory to our findings (18,19). Table 4 shows the salient features of some important studies reported in literature.

Table 4. HLA Class II association with Asthma and Serum IgE as reported by others.

Year	No of patients	Association with Asthma, Population Studied	Association with High Total Immunoglobulin E	Ref.
2002	57	DR7, DQA1*02:01, DQB1*02:02 Hungarians; Negative with DR4 - DQA1*03:01 - DQB1*03:02	Not studied	10
2005	263	DRB1*04 and DQA1*03:01 Greek	HLA-DQB1*03:01-04	13
1996	55	DR4, DR2,DR4; Negative with DR7 and DQ2 Greek	Not studied	14
1999	64	DRB1*15:01/:02 Shanxi, China	Not studied	19
2008	112	DRB1*12, DQB1*06:03,1*06:04 Iranian	DQB1*0301, DQA1*0505 – Iranian (21)	21
	103	DRB1*04, DQB1*03, DQB1*02;02 (Asian Indian) negative with DRB1*15	DRB1*14, but not statistically significant	Current study

A study on Japanese children showed a positive association with DPB1*09:01 with asthma (20). However, we did not find any association with DPB1 alleles which could be attributed to the low frequency of the allele in population studied small sample size and ethnic variations.

Strengths of our study include the fact that it was performed on children with a defined disease who had been under regular follow up for two to three years with confirmation of diagnosis by clinical and lab work up including pulmonary function tests for compliant children.

Mohadvedi *et al*. found a positive association of DRB1*12, DQB1*06 with pediatric

asthma in the first ever study in Iran, whereas a Venezuelan study pointed towards DRB1*11 as a susceptibility factor for asthma (21,22). Table 4 shows a comparison of our findings compared with some others reported in literature.

A study by Borish *et al.* showed patients with severe or difficult-to-treat asthma had higher total IgE levels, particularly so in males, children, smokers, non-white racial/ethnic groups, and adults with childhood-onset disease (23). However our study did not show such association which could also be due to very small percentage of patients with severe asthma (6%) in our study and an accurate inference may be derived on the basis of a study with larger proportion of severe asthma.

This study also brought out the diversity in class II antigens. There were DR 15, DR4 and DR7 associated with DQ5; DR4 and DR14 with DQ6 and DR1 with DQ2. This is relevant because these are probably less well described associations of DRB1 and DQB1 antigens. Considerable heterogeneity was observed in DPB1 alleles where 34 different DPB1 alleles were identified in 94 samples.

In conclusion, the difference in allele association with pediatric asthma in different populations may be dependent on their ethnicity and environmental exposure. The study showed a positive association of DRB1*04/DQB1*03 alleles and DQB1*02:01 allele with extrinsic asthma and a negative association with DRB1*15 allele in Indian children. However, more studies are required on larger populations to confirm the association of HLA Class II alleles in Indians before a particular allele can labeled as being protective or causative for asthma.

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