

BRIEF COMMUNICATION

A study of the association of childhood asthma with HLA alleles in the population of Siliguri, West Bengal, India

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Key words

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Abstract

Asthma is a heterogeneous disease for which a strong genetic basis is firmly established. It is a complex disorder influenced by gene–environment interaction. Human leukocyte antigen (HLA) genes have been shown to be consistently associated with asthma and its related phenotypes in various populations. The aim of this study was to determine the frequency of the selected HLA classes I and II allelic groups in asthmatic and control groups. HLA typing was performed using polymerase chain reaction–sequence-specific typing (PCR–SSP) method. The allele frequency was estimated by direct counting. Frequency of each HLA allelic group was compared between asthmatic group and control group using χ^2 test. *P*-value was corrected by multiplying with the number of the allelic groups studied. Odds ratio (OR) and its corresponding 95% confidence interval (CI) for each allelic group were calculated using GRAPHPAD INSTAT 3.10. The results of this study showed a significantly higher frequency of *HLA-DRB1*03* in asthmatics than in controls (11.43% vs 3.64%, OR = 3.78, 95% CI = 1.61–8.85, *P* = 0.0025, *P*_{corr} < 0.05). Analysis of HLA alleles in low and high total serum immunoglobulin E (IgE) level in asthmatics revealed no significant association. *HLA-DRB1*03* may be implicated in the susceptibility to asthma in the pediatric population.

Introduction

Asthma is a common chronic inflammatory disease of the conducting airways which undergo distinct structural and functional changes, leading to non-specific bronchial hyper-responsiveness (BHR) and airflow obstruction that fluctuates over time (1). It is a complex disease determined by many genes and molecular mechanisms. Indeed, the genetic factors contribute to disease susceptibility but the manifestation of the disease is modulated by environmental exposures and the interactions between both of these components. Candidate-gene and linkage studies followed by positional cloning have already provided a large number of genes accountable for the susceptibility to asthma (2). In addition, many genome-wide association studies (GWASs) published in recent decade have identified several genetic loci to be associated with asthma and/or its related phenotypes in different populations (3–5).

The major histocompatibility complex (MHC), designated as human leukocyte antigen (HLA) in human, genes map on chromosome 6p21 play an important role in the regulation of the immune system (6). Many studies have documented that

6p21 region is strongly linked to atopic phenotype and asthma and it is considered a major locus influencing allergic diseases (7–9). Further, numerous studies have investigated the association of HLA alleles and/or antigens with asthma. Some of the earlier studies have reported the association of various HLA class I alleles/antigens (10, 11), while large number of studies have investigated the association of HLA class II alleles/haplotypes with asthma in different populations. Choi et al. reported a strong association between *HLA-DPB1*0301* and aspirin-intolerant asthma in Korean population (12). In another study, Movahedi et al. reported the association of HLA class II alleles with asthma and high total IgE levels in Iranian children (13). Lara Marquez et al. have reported the association of HLA class II alleles and haplotypes with *Dermatophagoides species* sensitive asthmatics in Venezuelan population (14). Similarly, Guo et al. also reported the association of HLA-DQ genes with asthma and positive specific IgE response to *Dermatophagoides species* in Chinese population (15). In a study on Korean population, Kim et al. have reported the association of HLA haplotype with isocyanate-induced occupational asthma (16). Another study on Greek children with allergic asthma revealed the association of *HLA-DRB1*04*

and *DQA1*0301* with susceptibility to mites sensitive asthma (17).

Siliguri is located in the foothills of the Himalayas. It is a city in the Indian state of West Bengal and known as the gateway to North East India, Bhutan, Nepal and Bangladesh. The adjoining areas of Siliguri are comprised of tea gardens and villages. People of diverse communities are the inhabitants of this region. The majority of population belongs to Bengali community while other minority communities include Nepali, Marwari, Bihari and native tribal community (<http://en.wikipedia.org/wiki/Siliguri>. Accessed on 16.1.2014). There is a paucity of data particularly on HLA association with asthma in pediatric population of this region. Therefore, the purpose of this study was to determine the frequencies of some of the selected HLA classes I and II allelic groups in asthmatic and control subjects.

A total of 105 unrelated asthmatic children, aged 3–12 years, were recruited in this study from the Out Patient Department of Pediatrics, North Bengal Medical College & Hospital, Siliguri, West Bengal, India. Asthma was diagnosed by a physician based on the physical examination, clinical symptoms, response to bronchodilators, etc. Age and sex matched 110 unrelated children of the same ethnic background were included as controls. The criteria for the selection of control subjects included: no history of allergy, airway hyper-responsiveness, upper respiratory tract infections and lung disease. The study was approved by the Institutional Ethics Committee, University of North Bengal. The written informed consent was obtained from the parents/guardians for their children to participate in the study. The blood samples were collected under appropriate conditions by vein puncture method and the demographic and clinical characteristics of the participating subjects were collected using the questionnaire.

Genomic DNA was extracted from the venous blood using Phenol-Chloroform method. Molecular typing of the selected HLA classes I and II allelic groups was performed by polymerase chain reaction using sequence-specific primers (PCR-SSP). We studied the allelic groups which have been reported previously to be associated with childhood asthma in various populations (10, 11, 13, 17–25). The primer sequences of HLA class I allelic groups were taken from Bunce *et al.* (26) and of class II allelic groups were obtained from Zhu *et al.* (27) and Sacchetti *et al.* (28). The primers were synthesized and supplied by Integrated DNA Technologies (IDT).

A 25 µl reaction mix was prepared and the reaction was carried out in a thermal cycler (MJ MiniTM Gradient Thermal Cycler, PTC-1148, Bio-Rad, Singapore). Touchdown PCR was adopted for the amplification of the DNA with the following reaction conditions: Initial denaturation at 94°C for 3 min followed by five cycles of 94°C denaturation for 30 s, 2°C higher annealing temperature (varying for different alleles) for 35 s and 72°C extension for 40 s, and 25 cycles of 94°C denaturation for 30 s, 2°C lower annealing temperature for 50 s and 72°C extension for 1 min. A final extension at 72°C for 7 min

Table 1 Characteristics of asthmatic and control subjects

	Asthmatics	Controls
No. of subjects	105	110
Males	57	58
Females	48	52
Age (years): mean ± SD	7.33 ± 2.62	7.71 ± 2.74
Age of onset		
Before 5 years	85	
After 5 years	20	
Height (cm): mean ± SD	116.35 ± 13.28	117.63 ± 13.03
Weight (kg): mean ± SD	19.19 ± 5.94	20.95 ± 6.28
Study groups		
Bengali	78	69
Bihari	12	15
Nepali	7	15
Others	8	11

SD, standard deviation.

was performed. In order to avoid technical error in amplification, two different internal controls were used. An internal control (hemoglobin gene) of 256 bp was used for typing of class I allelic groups and another internal control (a fragment of human growth hormone gene1) of 439 bp was used for class II allelic groups. The PCR products were electrophoresed in 2% prestained agarose gel. We also analyzed the association of HLA allelic groups with the elevated level of total serum immunoglobulin E (IgE) in asthmatic subjects. Available data of total serum IgE of 70 asthmatic subjects of our earlier study were used for this purpose (29).

The frequencies of the HLA allelic groups were determined by direct counting. The frequency of each allelic group observed in the asthmatic group was compared to control group using χ^2 test. *P*-values were corrected (P_{corr}) by multiplying with the number of allelic groups studied. A *P*-value <0.05 was considered to be statistically significant. The odds ratio (OR) with its corresponding 95% CI for each HLA allelic group was calculated using the GRAPH PAD IN STAT version 3.10.

The characteristics of asthmatic and control subjects are summarized in Table 1. The result showed the higher frequencies of *A*01* (15.71% vs 10.45%), *A*03* (17.62% vs 14.09%), *A*24* (16.67% vs 13.64%), *A*26* (12.86% vs 8.64%), *B*08* (21.90% vs 18.64%), *B*37* (19.05% vs 15.45%), *DRB1*01* (6.67% vs 3.18%), *DRB1*03* (11.43% vs 3.64%), *DQB1*0201* (18.57% vs 14.09%), *DQB1*0302* (14.29% vs 10.00%) and *DQA1*0501* (14.76% vs 10.91%) and the lower frequencies of *A*11* (8.57% vs 10.00%), *B*51* (6.19% vs 8.18%), *B*52* (4.29% vs 5.91%), *DRB1*04* (15.24% vs 18.18%) and *DQB1*0603/8* (2.86% vs 4.55%) in asthmatic group than in controls, respectively (Table 2). Among these, the frequency of *HLA-DRB1*03* was significantly higher in asthmatics than in controls (11.43% vs 3.64%, OR = 3.78, 95% CI = 1.61–8.85, *P* = 0.0025, *P_c* < 0.05) while the frequencies of rest of the allelic groups did not show significant difference between the two groups. When the frequencies of *HLA-DRB1*03* were compared between asthmatic

Table 2 Frequencies of HLA class I and class II allelic groups in asthmatic and control subjects^a

HLA allelic group	Asthmatics (N = 105) Freq. % (n)	Controls (N = 110) Freq. % (n)	χ^2	P	OR (95% CI)
A*01	15.71 (33)	10.45 (23)	2.564	0.109	1.73 [0.94–3.21]
A*03	17.62 (37)	14.09 (31)	0.932	0.334	1.39 [0.78–2.47]
A*11	8.57 (18)	10.00 (22)	0.132	0.717	0.83 [0.42–1.65]
A*24	16.67 (35)	13.64 (30)	0.670	0.413	1.33 [0.74–2.39]
A*25	10.48 (22)	9.09 (20)	0.116	0.734	1.19 [0.61–2.34]
A*26	12.86 (27)	8.64 (19)	1.802	0.179	1.66 [0.86–3.21]
B*08	21.90 (46)	18.64 (41)	0.701	0.403	1.31 [0.76–2.26]
B*44	5.71 (12)	3.64 (8)	0.662	0.416	1.65 [0.64–4.20]
B*45	7.14 (15)	5.00 (11)	0.569	0.451	1.50 [0.65–3.44]
B*51	6.19 (13)	8.18 (18)	0.406	0.524	0.72 [0.33–1.56]
B*52	4.29 (9)	5.91 (13)	0.314	0.575	0.70 [0.29–1.71]
B*37	19.05 (40)	15.45 (34)	0.931	0.335	1.38 [0.78–2.42]
DRB1*					
01	6.67 (14)	3.18 (7)	2.223	0.136	2.26 [0.88–5.85]
03	11.43 (24)	3.64 (8)	9.106	0.0025 ^{b,c}	3.78 [1.61–8.85]
04	15.24 (32)	18.18 (40)	0.593	0.441	0.77 [0.43–1.35]
12	8.57 (18)	5.45 (12)	1.258	0.262	1.69 [0.77–3.71]
DQB1*					
0201	18.57 (39)	14.09 (31)	1.578	0.209	1.51 [0.85–2.67]
0302	14.29 (30)	10.00 (22)	1.710	0.191	1.60 [0.85–3.01]
0603/8	2.86 (6)	4.55 (10)	0.467	0.495	0.61 [0.21–1.73]
DQA1*					
0501	14.76 (31)	10.91 (24)	1.295	0.255	1.50 [0.81–2.78]

CI, confidence interval; HLA, human leukocyte antigen; OR, odds ratio.

^a% = (n/2N) × 100.^bP corrected < 0.05.^cFisher exact = 0.0018.

Bengali and control Bengali groups to exclude the possibility of significant influence of population admixture, it was found significantly higher frequency of *HLA-DRB1*03* in asthmatic Bengali group than in control Bengali group (13.16% vs 3.62% respectively; OR = 4.41, 95% CI = 1.56–12.52, $P = 0.006^{**}$). Although the *HLA-DRB1*01* allelic group was not significantly associated with asthma, the OR for this allele was greater than 2 (OR = 2.26, 95% CI = 0.88–5.85, $P = 0.136$). Further analysis of the frequencies of HLA allelic groups in two groups of asthmatic subjects viz. tIgE > 150 IU/ml and tIgE < 150 IU/ml showed the higher frequencies of A*01 (18.0% vs 12.5%), A*24 (17.0% vs 12.5%), A*25 (12.0% vs 7.5%), B*52 (5.0% vs 2.5%), B*37 (19.0% vs 15.0%), DRB1*01 (9.0% vs 5.0%), DRB1*03 (11.0% vs 7.5%), DQB1*0302 (16% vs 10.0%) and DQA1*0501 (15.0% vs 10.0%), and the lower frequencies of A*03 (14% vs 17.5%), A*26 (13% vs 15%), B*08 (20.0% vs 22.5%), DRB1*12 (8.0% vs 12.5%) and DQB1*0201 (17.0% vs 20.0%), respectively. None of these showed the significant difference between the two groups.

In this study, it was observed that the frequency of *HLA-DRB1*03* was significantly higher in asthmatic subjects than in controls (OR = 3.78, 95% CI = 1.61–8.85, $P = 0.0025$, $P_c < 0.05$). The association of *HLA-DRB1*03* with childhood asthma, in this study, is consistent with the findings of various earlier studies. A study conducted by Ivković-Jureković *et al.* in Croatian asthmatic children, showed a significantly

higher frequency of *HLA-DRB1*03* specificity among the asthmatic patients with total serum IgE ≥ 400 kU/l (30). In another study, Juhn *et al.* reported that *HLA-DRB1*03* allele was the most significantly associated with an increased risk of asthma (HR: 1.5, 95% CI: 1.0–2.4, $P = 0.050$) (23). Similarly, Hanchard *et al.* suggested the role of *HLA-DRB1*03* in asthma susceptibility independent of ancestral-haplotype-mediated linkage disequilibrium (31). In addition, Rajagopalan *et al.* have reported that *HLA-DRB1*03* allele plays an important role in determining the eosinophilic airway inflammation, a Th2 mediated inflammation (32).

The frequency of *HLA-DRB1*01* was higher in asthmatic subjects than in controls, although the difference was not significant (6.67% vs 3.18%, respectively; OR = 2.26, 95% CI = 0.88–5.85, $P = 0.136$). Analysis of HLA alleles in association with the elevated level of total serum IgE showed that none of the HLA alleles was found to be associated with the elevated level of total serum IgE in asthmatic subjects. Many studies have reported the positive significant association of *HLA-DRB1*01* with the elevated level of total serum IgE in asthmatics and/or allergic patients. Torio *et al.* showed a significant association of DRB1*01 with the elevated level of total serum IgE in the Spanish Artemisia sensitive asthmatics (24). Woszczek *et al.* reported the significantly higher total serum IgE levels in allergic patients with *HLA-DRB1*01* compared to patients without these allele (25). Similarly,

Ulbrecht et al. reported a weak association of *HLA-DRB1*01* with specific IgE-positive cases compared to negative controls (33).

Asthma and its associated trait 'atopy' were some of the first complex diseases for which a strong genetic basis was established (34). HLA class II antigens play a key role in antigen presentation to CD4⁺ T-lymphocytes and therefore influence the specificity of the immune response. HLA genes have been implicated in triggering an allergen-specific IgE response. The amino acid constituents of the specific epitopes of allergens have been identified and specific HLA-DR and DQ gene products have been shown to present these epitopes (35). HLA-DR alleles are found to be associated with the development of specific IgE response to seasonal as well as perennial allergens (36, 37). Murray suggested the potential role of HLA genes in determining the Th1 vs Th2 immune response through the interaction between T-cell receptor, peptide and MHC molecules (38). Blumenthal et al. have suggested a different role of HLA gene polymorphism. They showed that in pollen allergy, the asthma phenotype may be associated with extended haplotype HLA-B7/SC31/DR2 (39).

Although it has been known that HLA is associated with asthma and/or related phenotypes but its exact role in disease pathogenesis is still not clearly understood. Probably, HLA alleles act in association with other genetic loci responsible for the regulation of total IgE. It has been shown that genetic loci of chromosome 11q and 5q are strongly associated with high total IgE levels (40, 41). Therefore, the regulation of the total IgE response is a complicated process involving several genetic loci.

In this study, we failed to establish the association of HLA alleles with high level of total serum IgE in asthmatic subjects. It could be because of the limitations of our study which include: relatively small number of subjects in total IgE < 150 IU/ml group of asthmatics. Further, we studied only limited number of allelic groups which have been reported previously to be associated with pediatric asthma in different populations. Therefore, further study in large cohort of asthmatic subjects of this region taking into account as many HLA allelic groups as possible is needed to support the present finding.

In conclusion, the present preliminary finding suggests the possible association of *HLA-DRB1*03* allelic group with asthma in the pediatric population of Siliguri region of West Bengal, India. Therefore, *HLA-DRB1*03* allelic group may be implicated in the susceptibility to childhood asthma.

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Conflicts of interest

The authors have declared no conflicting interests.

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