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Negative Association of *HLA-DRB1*11* and *HLA-DRB1*12* Alleles with Aeroallergy Patients Visiting Allergy Centre (NIH), Islamabad, Pakistan

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Authors' Contribution
FA, SA and ZA designed the study. MH and AH executed the experimental work, analyzed the data and wrote the article. FA took the samples and patients' history. SR statistically analyzed the data. NA and ZA supervised the study

Key words
Alleles, Asthma, Genetic linkage, Hypersensitivity, Immunoglobulin E.

ABSTRACT

Aeroallergens include dust, pollens, cotton and threshing dust particles. Prevalence of different allergic diseases in Pakistan is about 66.1% and every year, almost 2.4 million people get vaccine for allergy from Allergy Centre, National Institute of Health (NIH), Islamabad. Human Leukocyte Antigens (*HLA)* genes are located on the short arm of the chromosome number 6 and position 21 and the allele frequency is associated with allergy. The study was conducted to investigate the relationship between the aero-allergens and the frequency of *HLA-DRB1*11* and *HLA-DRB1*12* allele in aeroallergen sensitized individuals. Blood samples of aeroallergen sensitized individuals were collected at the Allergy Centre, NIH, Islamabad. Then samples were analysed at the Department of Microbiology and Molecular Genetics, University of the Punjab, Lahore during the study period expanded from August 2014 to February 2015 for the presence of *HLA-DRB1*11* and *HLA-DRB1*12* allele using polymerase chain reaction (PCR). While total level of Immunoglobulin E (IgE) was measured by enzyme linked immunosorbent assay (ELISA) in all serum samples. This study indicated that the frequency of both alleles *HLA-DRB1*11*and *HLA-DRB1*12* in aero allergy patients is less as compared to healthy controls. *HLA-DRB1*11* demonstrated significant association with aeroallergens and could have a protective role for allergy while *HLA-DRB1*12* did not show any association with aeroallergen sensitization.

INTRODUCTION

When the substances in the environment that are harmless to the majority of the people react with the immune system of the susceptible person, allergy occurs; these environmental substances are called allergens. Some of the examples of the allergens are pet danders, pollens, insect's antigens, molds, food particles, house dust mite, *etc*. (Breiteneder and Chapman, 2014; Host *et al*., 2003; Price *et al*., 2006). The substances which are air born are called aeroallergens. Allergic rhinitis, bronchial asthma, urticaria, atopic eczema and allergic conjunctivitis are the main complications related to the aero-allergens (Yalcin *et al*., 2013; Renaudin *et al*., 2012; Greiner *et al*., 2011).

Allergic sensitization is the result of interaction between environmental allergens and genetic factors such as human leukocyte antigens (*HLA*) which present allergens for immune recognition and sensitization. *HLA* are the most polymorphic genes; this system of the genes and its alleles are responsible to display proteins on the cell surface for identification of allergens and regulation of immune system in human beings (Kindt *et al*., 2007). Its genes or gene products might be a risk factor or a protective factor for the allergy by common allergens (Agarwal, 2011; Ramasamy *et al*., 2011; Muro *et al*., 2013; Portelli *et al*., 2015).

Increased frequency of *HLA DRB*12* and *HLA-DRB1*03* has been reported in asthma patients in Pakistan and pediatric asthmatics of Indian population respectively (Javaid *et al*., 2014; Lama *et al*., 2014). *DRB1*10, DQB1*05* and *DQB1*602* reported as linked with rheumatoid arthritis (autoimmune disease) while *DRB1*07-DQB1*02* and *DRB1*11-DQB1*0301* play protective role (Muazzam *et al*., 2013). *HLA-DQB1*0302* and *HLA-DRB1*04* are

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interrelated with aspirin exacerbated respiratory disease (Esmaeilzadeh *et al*., 2015).

A number of *HLA* alleles have been investigated for its correlation among asthma, rhinitis, conjunctivitis and immunoglobulin E (IgE) levels with varying findings. Identification of particular allele responsible for allergy with particular association to risk factor might lead to prior allergy identification and preventive interventions. This study was conducted to evaluate the association of different aero-allergens and allergic complications like bronchial asthma, allergic rhinitis, allergic conjunctivitis, atopic eczema and allergic dermatitis, with two different alleles of *HLA-DRB1*, *i.e. HLA-DRB1*11* and *HLA-DRB1*12*.

PATIENTS AND METHODS

It is a comparative case-control study that comprised of 110 aeroallergen sensitized patients and 40 healthy controls**.** Sample size was calculated by Fleiss (1981) formula for un-matched case control study, keeping the power of study equal to 80% and level of significance equal to 5% (Fleiss, 1981; Park *et al*., 2012). Calculated sample size was 110 per group but because of financial constrains control group was reduced to 40 individuals (power of study=50%). The study was conducted at the Department of Microbiology and Molecular Genetics, University of the Punjab, Lahore during the time period of August 2014 to February 2015 after getting the approval from Institutional Biosafety and Bioresource Committee. The Blood samples were collected from Allergy Centre, National Institute of Health (NIH), Islamabad after confirmation by skin prick test (SPT), standardized by the NIH Allergy Center, Islamabad. Samples positive to SPT with pollen, paper mulberry, house dust mite, thrashing dust particles and raw cotton, of age 14-60 years of either gender were taken as aero-allergic samples and samples with negative result to SPT and no sign and symptoms for allergic reaction were taken as healthy controls. History of the sampling person was also recorded. Samples with rheumatoid arthritis, diabetes, multiple sclerosis, cancer or any autoimmune disease were excluded from the study. Serum was isolated from the blood samples and their total IgE level was estimated using BIOCHECK, Inc. Enzyme Immuno-sorbent assay for the Quantitative Detection of concentration IgE in serum samples of human (Biocheck, Inc, BC1035, US) according to the manufacturer's instruction and OD was taken at 450 nm.

DNA isolation was done with FAVORGEN FavorPrep Blood Genomic DNA Extraction Mini Kit (Favorgen, FABGK-001, Taiwan) according to manufacturer's instruction and was confirmed by gel electrophoresis (Sambrook and Russell, 2001).

HLA typing was performed for *HLA-DRB1*11* and *HLA-DRB1*12* alleles by using the technique SSP-PCR following the protocol of Olerup and Zetterquist (1992). Primers were synthesized by e-oligos. Primers for *HLA-DRB1* were used as described by Olerup and Zetterquist (1992) and for internal control GAPDH, the primers used were described by Paulukat *et al*. (2001). PCR optimization was performed using touchdown PCR. Primers for *HLA* alleles and internal control GAPDH were used. The primer sequences are given as follows:

Primers for *HLA-DRB1*11* (Olerup and Zetterquist, 1992)
Forward: 5'GTTTCTTGGAGTACTCTACGTC3'
Reverse: 5'CTGGCTGTTCCAGTACTCCT3'

Primers for *HLA-DRB1*12* (Olerup and Zetterquist, 1992)
Forward: 5'AGTACTCTACGGGTGAGTGTT3'
Reverse: 5'CACTGTGAAGCTCTCCACAG3'

Primers for GAPDH (internal control) (Paulukat *et al*., 2001)
Forward: 5'ACCACAGTCCATGCCATCAC3'
Reverse: 5'TCCACCACCCTGTTGCTGTA3'

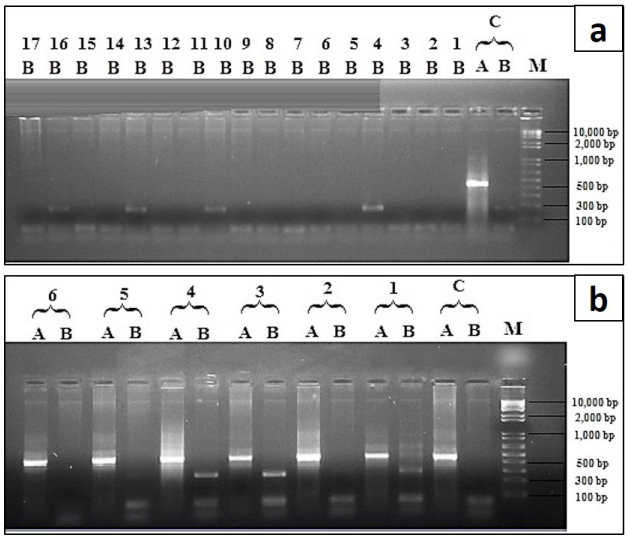

Fig. 1. HLA typing using PCR. M, DNA 1kb marker; C, positive controls for allele of interest; A, PCR with primers of internal control GAPDH (PCR product size = 576bp); B, PCR with primers of allele of interest; Allele of interest in a, *HLA-DRB1*11* (PCR product size = 176bp); Allele of interest in b, *HLA-DRB1*12* (PCR product size = 248bp); Lanes 1 to 17, Samples of aero-allergic patients.

Data was analyzed using SPSS 20.0. Mean ± S.D was calculated for quantitative variables and Frequencies and percentages were calculated for qualitative variables. Data distribution was tested by Shapiro-Wilk Test and Kolmogorov-Smirnov test. For normal data distribution, Student t-test was applied and for non-normal data

distribution Mann-Whitney test was applied. For statistical significance, p-value of ≤0.05 was considered as significant. Odds ratio was calculated and conventional confidence interval of 95% was used.

Table I.- Comparison of aeroallergen sensitized patients and Healthy controls (non-atopic) group for different parameters of the recruited persons.

Character-istics	Atopic (n=110)	Non atopic (n=40)	p-Values	Odd's Ratio CI (95%)
Gender, n (%)				
Male	107 (97.3%)	21 (52.5%)	p<0.0001	1.85 (1.37-2.49)
Female	3 (2.7%)	19 (47.5%)		
χ^2 Statistics	P = 0.32	P<0.0001		
Age (years)				
Mean (SD)	30.41 (10.70)	34.03 (13.27)		
Median	29.00	30.50		
Province, n (%)				
AJK	6 (5.5%)	0 (0%)	p<0.0001	
Baluchistan	1 (0.9%)	0 (0%)		
Federal	19 (17.3%)	0 (0%)		
KPK	26 (23.6%)	1 (2.5%)		
Punjab	55 (50.0%)	39 (97.5%)		
Sindh	3 (2.7)	0 (0%)		
Number of positive SPT				
Mean (SD)	3.78 (0.64)	0	p<0.0001	
Median (range)	4.00 (2-5)	0		
***HLA-DRB1*11*, n (%)**				
Positive	15(13.6%)	16 (40%)	p<0.0001	0.34 (0.18-0.62)
Negative	95 (86.4%)	24 (60%)		
***HLA-DRB1*12*, n (%)**				
Positive	22 (20.0%)	11 (27.5%)	p=0.033	0.72 (0.38-1.36)
Negative	88 (80.0%)	29 (72.5%)		

*CI, confidence interval; n, number; %, percentage; SD, standard deviation.

RESULTS

The aero-allergic patients recruited for study have the mean age of 30.41±10.70 with median age of 29 years and healthy controls have the mean age of 34.03±13.27 with median age of 30.5 years (Table I). The concentration of IgE in blood serum of aeroallergen sensitized individuals was 559.20 IU/ml (8.9-1486 IU/ml), while the non-allergic controls have IgE levels at 150.95 IU/ml (2.4-890 IU/ml).

HLA typing for HLA-DRB1*11 and HLA-DRB1*12 alleles was performed by using SSP-PCR as shown in Figure 1. Out of 150 recruited candidates, 16(40%) healthy controls were positive for *HLADRB1*11*, and 15(13.6%) were positive among aeroallergen sensitized patients (Fig. 2). The results were statistically significant (p=0.05), which indicates a negative association between *HLA-DRB1*11* allele with aero-allergy. However, odds of *HLA-DRB1*11* positive samples were 66% lower than *HLA-DRB1*11* negative individuals.

In case of *HLA-DRB1*12*, 11(27.5%) were positive among healthy controls and 22(20%) were positive among aeroallergen sensitized individuals. The results were statistically significant (p=0.03), which means there is a negative association between *HLA-DRB1*12* allele with aero-allergy (Fig. 2). The odds of *HLA-DRB1*12* positive samples was 28% lower than *HLA-DRB1*12* negative individuals (Table I).

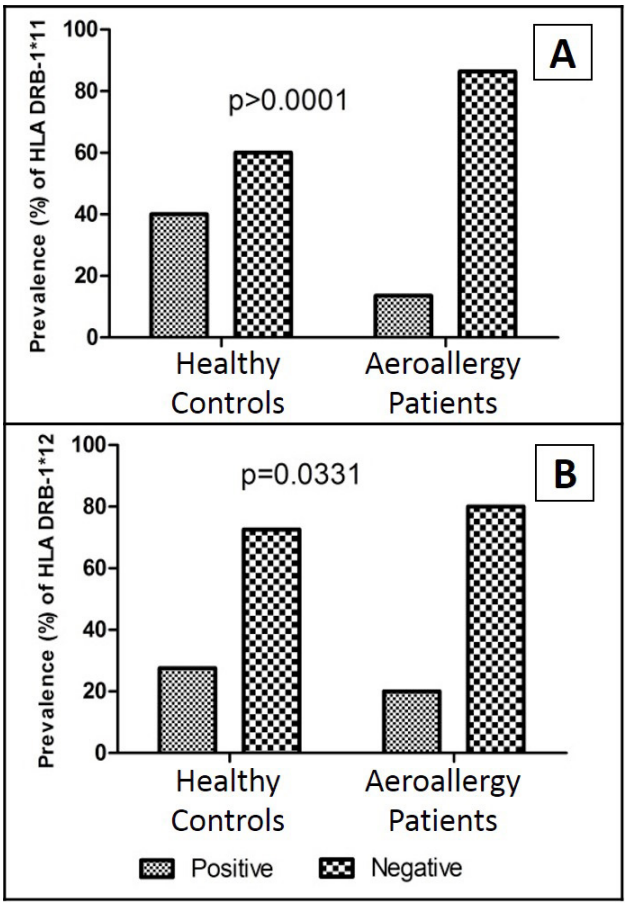

Fig. 2. Percentage prevalence of *HLA-DRB1*11* (A) and *HLA-DRB1*12* (B) amongst aero-allergy cases reported at Allergy Centre, NIH, Islamabad and healthy controls.

In this study, no significant association between *HLA-*

*DRB1*11* positive allele (p = 0.91) and *HLA-DRB1*12* positive allele (p = 0.95) with different provinces of Pakistan was found (Table II). While, the prevalence of *HLA-DRB1*11* and *HLA-DRB1*12* alleles varied widely among population of different provinces of Pakistan but highest was reported in Punjab and KPK.

The relationship of family history of allergy with both *HLA-DRB1*11* and *HLA-DRB1*12* was determined. The outcomes relate a significant relationship between family history and *HLADRB1*11* (p=0.05) but there is no significant association with *HLA-DRB1*12* (p=0.15) (Table III).

Association of different allergic diseases with both alleles was investigated by t-test and the outcomes show that there is no significant association of both the *HLA-DRB1*11* and *HLA-DRB1*12* alleles with the different atopic disorders (Table IV).

Table II.- Association of the *HLA-DRB1*11* and *HLA-DRB1*12* with patients residing in different provinces of Pakistan in the aeroallergen sensitized group.

Province	*HLA-DRB1*11*		*HLA-DRB1*12*	
	Positive	Negative	Positive	Negative
Total	15(13.6%)	95(86.4%)	22(20.0%)	88(80.0%)
AJK	1(6.7%)	5(5.3%)	1(4.5%)	5(5.7%)
Baluchistan	0(0.0%)	1(1.1%)	0(0.0%)	1(1.1%)
Federal	2(13.3%)	17(17.9%)	4(18.2%)	15(17.0%)
KPK	4(26.7%)	22(23.2%)	4(18.2%)	22(25.0%)
Punjab	7(46.7%)	48(50.5%)	12(54.5%)	43(48.9%)
Sindh	1(6.7%)	2(2.1%)	1(4.5%)	2(2.3%)
p-Values χ^2 Statistics	p = 0.918		p = 0.954	

Table III.- Association of the *HLA-DRB1*11* and *HLA-DRB1*12* with the cases with family history in the case group of the study.

Family History	*HLA-DRB1*11*		*HLA-DRB1*12*	
	Positive	Negative	Positive	Negative
Yes	11(73.3%)	42(44.2%)	14(63.6%)	39(44.3%)
No	4(26.7%)	53(55.8%)	8 (36.4%)	49(55.7%)
p-Values (χ^2 Statistics)	p = 0.05		p = 0.15	
Odd's Ratio (95%CI)	3.47 (1.03-11.68)		2.19 (0.83-5.77)	

DISCUSSION

Mid-ages are more prone to aeroallergen sensitization as compared to children and old aged persons as revealed by the results of present study. Govaere *et al*. (2007) stated that median age of 30 years is most suitable for sampling of allergy patients. The allergy prevalence remains different for the two genders before the age of 8 years, after which, the atopic behavior does not differ significantly. However, Niemeijer and de Monchy (1992) reveals that skin reactivity to histamines give constant results in the age group between 20 to 75 years.

In this study, the IgE levels were higher in aeroallergic individuals compared to non-aero allergic samples. According to Wahn (2014), serum IgE level is raised in the case of allergy thus supporting the results of present study.

Table IV.- Association of the *HLA-DRB1*11* and *HLA-DRB1*12* with the atopic diseases in the aeroallergen sensitized patients.

Diseases	n	*HLA-DRB1*11*		*HLA-DRB1*12*	
		Pos (15)	Neg (95)	Pos (22)	Neg (88)
		p-Values t statistics		P-Values t statistics	
Seasonal allergic rhinitis	25	0.35		0.25	
Allergic rhinitis	44	0.26		0.38	
Allergic conjunctivitis	17	0.60		0.79	
Bronchial asthma	21	0.13		0.90	
Urticaria	4	0.42		0.80	
Eczema	1	0.69		0.61	
Dermatitis	13	0.84		0.77	
Sinusitis	4	0.50		0.80	

n, total number of patients positive to disease.

Negative association of *HLA-DRB1*11*was found in aeroallergen sensitized individuals of the present study. According to an Iranian study, high frequency of *HLA-DRB1*11* allele is associated with raised IgE level in patients with asthma (Movahedi *et al*., 2008). Lara-Marquez *et al*. (1999) reported an association of *HLA-DRB1*11* allele with house dust mite atopic patients in Venezuelan population, while Slovak population not showed any association with *HLA-DRB1* gene with having highest frequency of *HLADRB1*11* to 16.1% (Lara-Marquez *et al*., 1999; Dzurilla *et al*., 2013). This difference in the frequency of *HLADRB1*11* indicates presence of different HLA haplotypes in population of different regions which could be directly associated with allergen. So, allergen susceptibility might be affected by different geographic regions, climate, and patient's lifestyle.

The results of this study exhibited lack of association of *HLA-DRB1*12* in aeroallergen sensitized individuals of Pakistan. There is a huge disparity in the reported results

regarding association of HLA-DRB1*12 with allergy in different populations. According to Movahedi *et al.* (2008) there is a strong association of *HLA-DRB1*12* with atopic individuals in Iranian population. A study showed association of *HLA-DRB1*12* with patients of chronic urticaria with high gene frequency in Chinese population (Chen *et al*., 2005). However, no association of *HLA-DRB1*12* was reported in asthma patients in the Venezuelan population and sensitization to pollen allergens like birch pollen (Juhlin *et al*., 1969; Sparholt *et al*., 1994). This drastic difference in association might be due to difference in allergen susceptibility of different populations. *HLA -DRB1*12* allele might function to produce its effects in respiratory tract to cause asthma in Iranian population, while in Venezuelan population; it might not produce significant products to cause asthma due to difference in allergen. Pollen allergen might not cause the change in the product of *HLA-DRB1*12* to cause allergy in Slovak community. While in Chinese population, allergens might affect *HLA-DRB1*12* allele to significantly produce active mediators to cause urticaria. The reason could be difference in epitope of allergen or its susceptibility in different regions.

NIH is situated in Islamabad and it is in close vicinity to residents of Punjab and Khyber Pakhtunkhawa (KPK). Sindh and Baluchistan regions are very far away from the NIH locality. In this study, the association was analyzed between alleles *HLA-DRB1*11* and *HLA-DRB1*12* with aero-allergic patients belonging to different provinces of Pakistan. Frequency of *HLA-DRB1*11* is different in different provinces of Pakistan and is highest in Punjab and KPK region. The reason for this high frequency is the higher prevalence of aeroallergens in Punjab and KPK province (Juhlin *et al*., 1969). Frequency of *HLA-DRB1*12* was also variable with the highest frequency found in Punjab that might be due to tropical forests in Punjab and high prevalence of pollen allergens (Ahmad *et al*., 2011). Therefore, no significant association between *HLA-DRB1*11* positive allele ($p = 0.91$) and *HLA-DRB1*12* positive allele ($p = 0.95$) with different provinces of Pakistan was found in this study.

The present study described a positive association of *HLA-DRB1*11* with family history of the patients with aero-allergy while negative association was observed in the case of allele *HLA-DRB1*12*. Wang *et al*. (2014) reported that there is a significant association of *HLA-DRB1*11* but no significant association of *HLA-DRB1*12* with family history. There was no significant association observed among both alleles to different allergic diseases in the present study. Our results are consistent with a study that reported no significant association of *HLA-DRB1*11* and *HLA-DRB1*12* with allergic rhinitis (Ahmad *et al*., 2011). Zicari *et al*. (2014) also reported a lack of association between allergic conjunctivitis and *HLA* polymorphism. Similarly, no significant association of these two alleles with house dust mite allergen and their lack of influence in causing dermatitis and eczema are reported somewhere. Movahedi *et al*. (2008) also demonstrates no significant association between asthma and *HLA-DRB1*12* and *HLA-DRB1*11* alleles. While Chen *et al*. (2005) reported positive association of chronic urticaria and *HLA-DRB1*12*.

The main limitation of the study was the inclusion of only 2 alleles for determining their relationship with the wide range of aero-allergen sensitizations. Therefore, we propose the study of some additional alleles in order to better understand the genetic associations of such genes with the various types of allergies and to make it clearer for the betterment in disease diagnosis and treatment.

CONCLUSION

*HLA-DRB1*11* allele has a relationship with aero-allergens. The frequency of gene is quite low but there is a significant association between the two, so, *HLA-DRB1*11* might play a protective role in the development of the allergy by different aero-allergens. On the other hand, no significant association between *HLA-DRB1*12* and aero-allergens was found. So *HLA-DRB1*12* allele is neither a significant risk factor nor a protective character in causing allergy. But both alleles confer susceptibility to the rise of blood serum IgE of the allergic subjects.

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Statement of conflict of interest

Authors declare that they do not have any conflict of interest regarding this study.

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