

## GENETIC ASPECT OF VENOM ALLERGY: ASSOCIATION WITH HLA CLASS I AND CLASS II ANTIGENS

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Karakış GP, Sin BA, Tutkak H, Köse K, Mısırlıgil Z: Genetic aspect of venom allergy: Association with HLA class I and class II antigens. *Ann Agric Environ Med* 2010, **17**, 119–123.

**Abstract:** Stings from bees and wasps can cause systemic allergic reactions in sensitized patients. However, the mystery of why some cases develop allergic reactions while others do not, remains poorly understood. We investigated whether particular human leukocyte antigen (HLA) class I and class II genes contribute to the development of venom allergy. A total of 21 bee and/or wasp venom sensitive patients who had life-threatening allergic symptoms after a sting, and positive diagnostic tests (Group 1), were included in the study. Thirty-seven healthy subjects without venom allergy (Group 2) were selected as the control group. HLA-class I (A-C) and class II (DR, DQ, DP) typing was performed by PCR-based techniques. HLA-B\*18 and HLA-Cw\*07 alleles was found more frequently in Group 1 than in Group 2 (14.3% vs 2.7%, p=0.026, and 31% vs 14.9%, p=0.036, respectively). Furthermore, HLA-A\*01 allele frequency had a trend to be higher in Group 1 than in Group 2 (14.3% vs 4.1%, p=0.055). However, the frequencies of HLA-A\*03 and HLA-Cw\*03 alleles were increased in Group 2 compared to Group 1 (20.3% vs 7.1%, p=0.049 and 10.8% vs 0%, p=0.024, respectively). Among HLA-class II genotypes, HLA-DQB1\*03 allele was significantly increased in Group 2 (60.9% vs 38.1%, p=0.018), while a higher frequency of HLA-DRB1\*03 and HLA-DRB1\*14 alleles showed a tendency statistically significant in Group 1 (9.5% vs 1.4%, p=0.057 and 11.9% vs 2.7%, p=0.058, respectively). HLA-B\*18 and HLA-Cw\*07 alleles may probably be associated with susceptibility to venom allergy, whereas HLA-A\*03, HLA-Cw\*03 and HLA-DQB1\*03 seem to be protective markers in a small Turkish population.

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**Key words:** genetic, human leukocyte antigens, hymenoptera, insect sting, venom allergy.

### INTRODUCTION

It is of interest that although many people have been exposed to bee and wasp stings, only a small number of subjects had anaphylactic reactions caused by venom allergens. Hymenoptera sensitivity occurring in families of patients with venom allergy have also been described [1, 2]. Additionally, the majority of beekeepers who are at increased risk of becoming allergic, do not produce specific IgE to bee venom, despite being stung very frequently [3].

In this context, we have hypothesized that an underlying genetic predisposition may be responsible for the development of abnormal immune response against venom allergens. An important candidate for such a disposition is the human leukocyte antigen (HLA) genes which play a major role in controlling the specific IgE immune response to allergens [4, 5]. Although an important association between allergic diseases and certain HLA class II alleles have been shown for several inhalant allergens [6, 7, 8, 9], few studies exist on histocompatibility antigens and IgE response to

bee or wasp venom. Lympány *et al.* [10], first demonstrated a protective association with HLA DR4 and DQw3, and specific IgE to mellitin, a major component of bee venom.

Therefore, the aim of this study is to identify the genetic properties for the development of venom allergy in the Turkish population. For this purpose, we performed HLA-class I and HLA-class II genotyping in patients with bee and/or wasp venom allergy, and healthy controls without venom allergy.

## MATERIALS AND METHODS

Between April 2001–2002, we investigated the HLA distribution of patients of Turkish Caucasian origin with venom allergy and controls without venom allergy. The study was approved by the local Ethics Committee of the School of Medicine at Ankara University. All study subjects were enrolled in the study after giving written informed consent.

**Patients.** Group 1 consisted of 21 individuals who attended the allergy clinic with a history of systemic allergic reactions to the hymenoptera sting. They all demonstrated positive skin testing to bee and/or wasp venoms, and elevated serum specific IgE levels. There were 4 females and 17 males with a mean age of  $35.7 \pm 8.5$  years (range: 18–53 yrs).

**Controls.** A total of 37 healthy subjects (Group 2) were selected for this study as the control group. They had no history of any local or systemic allergic reactions following bee or wasp sting, and negative skin test reaction to venoms was observed in all control subjects. The mean age was  $50.2 \pm 9.3$  years (range: 23–70 yrs, 31 males, 6 females). 18 individuals among them were normally exposed to stings and the remaining 19 were heavily exposed beekeepers. Beekeepers were randomly recruited from the population living in a rural area in Kızılcahamam, located at a distance of 100 km from Ankara, where beekeeping is an extremely popular practice. Despite the fact that they represent a risky population for development of venom allergy because of high exposure, none of them exhibited clinical evidence or a history of allergy (e.g. urticaria, angioedema, and/or anaphylaxis) to bee stings on multiple occasions. The duration of beekeeping ranged from 1–43 years (mean:  $24.8 \pm 13.4$  yrs). There were no related family members in the study groups.

**Assessment of venom allergy.** The same clinician evaluated all individuals, and a standardized questionnaire was applied in order to examine the detailed history of clinical symptoms after an insect sting. The diagnosis of bee and/or wasp venom hypersensitivity was confirmed by skin testings (prick and intradermal) and elevated titers of venom specific IgE antibodies in sera [11]. All patients and control subjects underwent skin testing with venom allergens by trained personnel in our laboratory. Skin prick tests were

performed with standardized pure venom extracts of the *Apis mellifera* and *Vespa vulgaris* (ALK-Abellò, Hørsholm, Denmark). Histamine dihydrochloride (10 mg/ml) and phenolated saline glycerol diluent were used as positive and negative controls, respectively. Venom specific IgE antibodies to bee and wasp venom were determined by using the CAP system (Pharmacia Diagnostics, Uppsala, Sweden) according to the manufacturer's recommendations. IgE antibody values greater than 0.35 kU/L were considered positive.

**HLA Typing.** HLA Typing was performed at the Immunology Laboratory of the Medical Faculty at Ankara University. Genomic DNA was extracted from 10 ml EDTA-peripheral blood lymphocytes by the use of a DNA isolation kit (Purogene Genomic DNA Purification Kit, Gentra Systems, USA). Polymerase chain reaction sequence-specific primer (PCR-SSP) techniques were used to analyze polymorphism at loci for human leukocyte antigen (HLA) class I A-B-C, HLA class II -DR, -DQ and HLA-DPA1, -DPB1. For this purpose, Olerup SSP™ HLA-A-B-C, HLA-DQ, -DR, HLA-DPA1 Combi Tray (Olerup SSP AB Saltsjöbaden, Sweden) and Dynal AllSet™ SSP HLA-DPB1 (Dynal Biotech. Ltd, UK) commercial kits were used according to the manufacturer's instructions [12]. Amplifications were carried out on a GeneAmp PCR System 9700 (Perkin Elmer Co., Warrington, UK).

**Statistical analysis.** SPSS for Windows 11.5 program (Chicago, USA) was used to analyze data. The frequencies of HLA-A, -B, -C and HLA-DR, -DQ, -DP alleles in the study groups were compared using Fisher's exact test and chi-squared test. A *p* value of less than 0.05 was considered to indicate a statistically significant difference between groups. Odds ratio (OR), and 95% confidence intervals (CI) were calculated.

## RESULTS

Clinical characteristics of the study population are summarized in Table 1.

**Group 1.** Classification of systemic allergic reactions was Grade III in 10 patients and Grade IV in 11 patients according to the method of Ring and Messmer [13]. 6 were sensitive to bee venom, 9 were sensitive to wasp venom, and the remaining 6 were sensitive to both bee and wasp venom. All patients had increased specific IgE levels to the related venom antigens. Specific IgE antibodies to *Apis mellifera* and *Vespa venoms* were  $10.7 \pm 6.5$  kU/L (range: 0.47–100) and  $16.2 \pm 8.3$  kU/L (range: 0.36–100) (mean  $\pm$  SEM), respectively.

**Group 2.** Both normally exposed subjects and also the beekeepers demonstrated negative skin test reactivity to bee and wasp venom antigens.

**Table 1.** Clinical characteristics of the study population.

	Venom allergic patients		Non-allergic control group	
			Beekkeepers	Healthy subjects
n	21		19	18
Sex (male/female)	17/4		19	12/6
Mean age + SD	35.7 ± 8.5		58.7 ± 8.5	41.2 ± 10.1
(Range) yrs	(18–53)		(40–70)	(23–57)
Duration of beekeeping	1–43 yrs (mean: 24.8 ± 13.4)			
Severity of reactions*	Grade III (n: 10) Grade IV (n: 11)			
Venom skin test sensitivity (n)	A.m. : 6 V.v. : 9 A.m. + V.v. : 6		Negative	Negative
Venom specific IgE	A.m. = 10.7 ± 6.5 kU/L (range 0.47–100) V.v. = 16.2 ± 8.3 kU/L (range 0.36–100)		ND**	ND**

A.m. – *Apis mellifera*; V.v. – *Vespa vulgaris*; \* – According to the Ring and Messmer's classification; \*\* – Not determined.

**Table 2.** Significant allele frequencies in the study population.

HLA	Control subjects (N=37) n (%)	Allergic patients (N=21) n (%)	OR	CI %	p
A*01	3 (4.1)	6 (14.3)	3.94	0.93–16.69	0.055
A*03	15 (20.3)	3 (7.1)	3.30	0.90–12.20	0.049
B*18	2 (2.7)	6 (14.3)	6.0	1.15–31.23	0.026
Cw*03	8 (10.8)	0 (0.0)	1.12	1.04–1.21	0.024
Cw*07	11 (14.9)	13 (31.0)	2.57	1.03–6.41	0.036
DQB1*03	39 (60.9)	16 (38.1)	2.54	1.14–5.65	0.018
DRB1*03	1 (1.4)	4 (9.5)	7.68	0.83–71.18	0.057
DRB1*14	2 (2.7)	5 (11.9)	4.86	0.9–26.29	0.058

OR – odds ratio; CI % – confidence interval.

**HLA Class I and Class II genes.** The results of all significant differences in allele group frequencies between the allergic patients and non-allergic control subjects are shown in Table 2. Odds ratios and 95% CI values were given in significant associations for comparisons between Group 1 and Group 2.

**HLA Class I Alleles.** The frequency of HLA-B\*18 and HLA-Cw\*07 alleles significantly increased in Group 1 when compared to Group 2 (for HLA-B\*18: 14.3% vs 2.7% p=0.026, OR: 6.0, 95% CI: 1.15–31.23 and HLA-Cw\*07: 31% vs 14.9% p=0.036, OR: 2.57, 95% CI: 1.03–6.41, respectively). In Group 2, HLA-A\*03 allele frequency was significantly higher than in Group 1 (20.3% vs 7.1% p=0.049, OR: 3.30, 95% CI: 0.90–12.20). Interestingly,

the frequency of HLA-A\*03 was significantly increased in the non-allergic beekeepers when compared to the allergic patients and non-allergic normal controls (p=0.004). However, it is noteworthy that HLA-Cw\*03 allele was completely absent among the Group 1, whereas the frequency was higher in Group 2 than in Group 1 (10.8% vs 0% p=0.024, OR: 1.12, 95% CI: 1.04–1.21). For HLA-A\*01 allele, the difference had a tendency to be significant after comparison between Group 1 and Group 2 (14.3% vs 4.1%, p=0.055 with OR: 3.94, 95% CI: 0.93–16.69).

**HLA Class II Alleles.** There was a significant increase in the frequency of HLA-DQB1\*03 allele in Group 2 when compared with Group 1 (60.9% vs 38.1%, p=0.018, OR: 2.54, 95% CI: 1.14–5.65). In contrast, the frequencies of HLA-DRB1\*03 and HLA-DRB1\*14 alleles were higher in Group 1 than in Group 2, showing a tendency to be statistically significant (for HLA-DRB1\*03: 9.5% vs 1.4%, p=0.057, OR: 7.68, 95% CI: 0.83–71.18 and HLA-DRB1\*14: 11.9% vs 2.7%, p=0.058, OR: 4.86, CI: 0.9–26.29, respectively).

No significant difference was found between study groups with respect to the frequencies of HLA-DPA1 and HLA-DPB1 alleles.

## DISCUSSION

It seems clear that IgE-mediated hypersensitivity to various antigens has been regulated by certain HLA class II alleles. Since the first study by Levine *et al.* [14], a body of evidence indicates the presence of HLA-DR and -DQ associations with several allergic situations [15, 16, 17, 18, 19]. However, the relationship between HLA genes and venom allergy has not been exhaustively studied.

Our results showed that HLA-A\*03, HLA-Cw\*03 and HLA-DQB1\*03 haplotypes was preferentially expressed in the non-allergic subjects compared to the allergic patients. This finding suggests that these particular HLA alleles may protect against the development of venom allergy in the non-allergic controls. Associations with class I alleles and allergy are less clear, as antigen processing and presentation via class I molecules have no direct implication for the recognition of allergenic epitopes. However, few studies looking at HLA class I alleles and allergy have been performed and their results are often inconclusive. Sanchez-Velasco *et al.* [20] have recently reported that the HLA-A\*01, HLA-B\*57 and HLA-B\*5901 Class I alleles were associated with risk to bee venom allergy, but we found that HLA-B\*18 and HLA-Cw\*07 haplotypes were more clearly over-represented in venom allergic patients than in controls without venom allergy. In accordance with their finding, venom allergic patients had a trend to be higher frequency for HLA-A\*01 allele in our study. This observation may indicate that the importance of haplotypes for susceptibility to bee or wasp venom hypersensitivity could vary among different ethnicity.

In a study by Lympany *et al.*, patients who were allergic to bee venom mellitin possessed a significantly reduced frequency of HLA-DR4 and DQw3 alleles compared with control subjects, suggesting a preventive effect. In their study, they found no significant differences in the frequency of HLA class II alleles between the bee-venom insensitive beekeepers and the normal control population who were not skin prick tested (10). Another study of patients sensitized to bee and wasp venom also reported positive associations with HLA-DRB1\*07 and DRB1\*11. In their studies, Faux *et al.* [21], did show a decreased prevalence of DRB1\*04 allele in wasp sensitive patients, suggesting that DRB1\*04 may represent a protective factor against disease in these patients. In our study, HLA class II alleles did not reach statistical significance when the *p* value was corrected. We did not find any association between HLA-DPA1, -DPB1 allele frequencies and IgE responses to venom allergens, in accordance with findings in a previous study [21].

It is noteworthy that in a study by Lympany *et al.* [10], patients had specific IgE to mellitin. Faux *et al.* [21], also recruited their patients according to the presence of specific IgE to the bee or wasp venoms. In the study of Sanchez-Velasco *et al.* [20], patients had a well documented bee venom phospholipase A<sub>2</sub> sensitivity, because allergic reactions to bee sting are known mainly due to major allergen of bee venom, phospholipase A<sub>2</sub>. Our patients were recruited because they demonstrated both positive skin test reactivity and high levels of specific IgE to whole bee and/or wasp venom. Additionally, they all had severe systemic allergic reactions following a sting. Therefore, there is a possibility that the different selection methods may account for the different HLA associations seen in several studies.

Because the observed differences in HLA distribution did not change according to the subjects' age and gender, we did not take this into consideration a possible source of bias [18, 19, 20, 21, 22].

Furthermore, venom specific IgE antibodies were not tested in the serum of controls as they had both negative allergy history to sting and negative skin test reactivity to venom allergens. Because beekeeping by humans is a common practice in Turkey, bee stings are not rare. In the majority of beekeepers, a varying number of bee stings have not resulted in allergic reaction. Non-allergic beekeepers who are skin test negative, give no history of any allergic reaction to bee venom, and have commonly negative sIgE [10]. Eich-Wanger *et al.* [23] also found lower skin test sensitivity and lower levels of venom-specific IgE while venom-specific IgG was higher, even in the allergic beekeepers.

This study contained 2 groups of subjects as non-allergic controls. In fact, the most appropriate control group consisted of beekeepers who were not allergic to bee and wasp venoms despite multiple stings. They represent a unique population for hymenoptera venom studies. Natural exposure to multiple bee stings in non-allergic beekeepers

have been demonstrated to induce tolerance to high doses of allergen [24]. The rationale behind the inclusion of this special population was that we wondered whether venom allergic patients were different from non-allergic beekeepers with regard to the HLA alleles for protection against allergy besides specific IgG4 antibodies and immune tolerance mechanisms.

Finally, allergic reactions caused by hymenoptera venoms represent a major medical problem for certain groups of the population. It is not known why all bee or wasp stings do not lead to anaphylactic responses. As mentioned above, a few studies suggest that genetic influences could be one important factor regulating type 1 allergy, which may predispose an individual to hymenoptera venom sensitivity and severe allergic symptoms. Although the sample size of our study is insufficient to answer the questions raised, our data reveals a difference in the ability of individuals with bee or wasp sting allergy and without allergy to respond to antigens involved in these reactions. Thus, it can be postulated that subjects bearing HLA-Cw\*07 and HLA-B\*18 alleles would be able to respond to bee or wasp venom common epitopes. In contrast, HLA-A\*03 allele which we have identified as increased in non-allergic beekeepers as compared to both allergic patients and non-allergic healthy controls, could be more protective against venom allergy. Because non-allergic beekeepers had tolerated bee stings on multiple occasions, whereas non-allergic normal subjects experienced no reaction after only one sting

In conclusion; this study provides further evidence for the possible role of genetically determined susceptibility in patients with venom allergy. We believe that an understanding of the genetic basis of allergic diseases is essential, not only to explain their pathophysiology, but also to prevent them.

#### Acknowledgements

This study was supported by the Ankara University Research Foundation, Grant N° 2003-08-09-124.

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