HLA-E*0101/0103X is Associated with Susceptibility to Pemphigus Vulgaris: A Case-control Study

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ABSTRACT Pemphigus vulgaris (PV) is a life-threatening, autoimmune blistering disease of the skin and mucous membranes. The relationship between PV and human leukocyte antigen (HLA) has been studied in several reports. Previous reports have demonstrated that HLA-E polymorphisms may have a role in the susceptibility to various autoimmune diseases. Our aim was to evaluate the role of HLA-E gene polymorphisms in the pathogenesis of PV in a Turkish population. A total of 49 patients with PV and 50 healthy subjects were enrolled into the study. We sequenced and analyzed the HLA-E gene from genomic DNA obtained from peripheral blood samples of the study groups. HLA-E haplotyping was performed by Sanger sequencing of PCR products of the HLA-E gene and HLA-E alleles determined by using SeqScape® software according to the World Health Organization (WHO) Nomenclature Committee for Factors of the HLA System. The frequency of the HLA-E*0101/*0103X genotype in male patients with PV was found to be significantly higher than in men in the control group (P=0.023). In addition, the frequency of the HLA-E*0103X/*0103X genotype was significantly lower in patients with PV than the control group (P=0.040). We also detected that the frequency of the HLA-E*0101/*0103X genotype in patients with mucocutaneous type PV and the frequency of the HLA-E*0101/*0101 genotype in patients with mucosal type PV was significantly higher than those in other types of PV (P=0.001 and P=0.006). The results of this study indicate that carrying the HLA-E*0101/0103X genotype may increase the risk of PV in male patients.

KEY WORDS: pemphigus, HLA-E, genetics

INTRODUCTION

Pemphigus is a group of rare and life-threatening autoimmune bullous diseases of the skin and mucous membranes, characterized by intraepidermal blistering. It is associated with pathogenic antibodies directed against desmoglein (Dsg) 3 and/or Dsg1, two transmembrane components of desmosomes, which are cell-cell adhesion complexes (1).

Pemphigus is a rare disease with an incidence ranging from 0.7-5 new cases/year per million. Pemphigus vulgaris (PV) is the main clinical subtype of the disease. PV often affects individuals between the ages 40 and 60, and its main characteristics are flaccid blisters and erosions of the mucous membranes and the skin. The most characteristic clinical findings are painful erosions and ulcers in the oral mucosa (2).

Genetic susceptibility to PV has gradually become an area of interest after the publication of two reports on the increased incidence of HLA-A10 in the Japanese

and Jewish populations (3,4). Certain human leukocyte antigen (HLA) class II alleles are more prevalent in patients with PV than in the general populations. Previous studies have demonstrated that HLA alleles DRB1*0402 and DQB1*0503 are positively associated with PV in multiple populations (5-7). Furthermore, some disease associations have been also reported with classical HLA class I (Ia) antigens, including HLA-A10, B35, B44, and CW4 (4,8-12).

In the Turkish population, significant associations with DRB1*04, DRB1*14, DQB1*05, DPB1*0401, CW*01, DR14, DQ8, DQ4, B35, B44, CW4, and PV have been demonstrated in three different studies (10,13,14).

Only a very limited number of studies has been published on non-classical HLA class Ib alleles (HLA-E, -F, -G) and PV, and only one of them on HLA-E and PV (15-17).

In humans, the location of the HLA-E genetic region is between the classical class I genes HLA-A and HLA-C on chromosome 6p21 (18). The HLA-E gene has been recently reported to have six alleles: HLA-E*0101, HLA-E*0103X (HLA-E*01031, HLA-E*01032), HLA-E*0104, HLA-E*0105, HLA-E*0106, HLA-E*0107 (19).

Regarding blistering diseases, a study from North America only pointed out that the homozygous genotype HLA-E*0103X/0103X is associated with susceptibility to PV in a cohort including Caucasian and Ashkenazi Jewish patients (17).

Herein we evaluate the potential association between PV and HLA-E polymorphisms in a Turkish cohort.

PATIENTS AND METHODS

Study Design

A prospective case-control study was performed to evaluate the potential association between PV and HLA-E polymorphisms. The patients and controls were matched for age and sex and recruited over a 12-month period. This study was conducted according to the principles of the Declaration of Helsinki, approved by the local ethics committee, and was supported by the Scientific Research Unit of Karadeniz Technical University under project number 1002.

Patients and controls

We aimed to enroll, consecutively, all patients with a diagnosis of PV who were admitted to the clinics of the Department of Dermatology at the Faculty of Medicine at Karadeniz Technical University in 1-year period. The following criteria was used for inclusion: (i) a confirmed diagnosis of PV with clinical, histological, and direct immunofluorescence findings, (ii) no additional autoimmune disorders, and (iii) informed consent.

Forty-nine patients with a diagnosis of PV (22 women and 27 men with a mean age of 53.3 years, range: 23-79) were enrolled in the patient group. The control group consisted of 50 healthy age- and sex-matched individuals (23 women and 27 men with a mean age of 53.2 years, range: 24-79). They had no known systemic diseases. Informed consent was also obtained. The source population for cases and controls was the same.

Collection of data and samples

All cases and controls were visited by a dermatologist MD who registered demographic, clinical, and laboratory findings and other relevant data on a case report form. Venous blood samples were taken with ethylenediaminetetraacetic acid (EDTA) tubes from each of the individuals in both groups.

Genomic DNA isolation from the samples

Genomic DNA of participants was isolated using the classical salting-out method (20).

Haplotyping of the HLA-E gene by Sanger Sequencing

The HLA-E gene (NM_005516) was amplified by polymerase chain reaction (PCR) using specific primers obtained by the Primer 3 program. Sequencing reactions were carried out by using BigDye® Terminator v3.1 Cycle Sequencing Kits and the ABI PRISM 3130 DNA analyzer (Applied Biosystems, Foster City, CA,

Table 1. The comparison of the frequencies of the human leukocyte antigen (HLA)-E genotypes between patients with pemphigus vulgaris and control subjects

	HLA-E*0101/*0101 (%)			HLA-E*0101/*0103X (%)			HLA-E*0103X/*0103X (%)		
	PV	Control	*P*	PV	Control	*P*	PV	Control	*P*
Men	11.1	11.1	1.000	81.5	48.1	**0.023**	3.7	40.7	**0.003**
Women	27.3	30.4	1.000	45.5	43.5	1.000	27.3	26.1	1.000
Total	18.4	20.0	1.000	65.3	46.0	0.084	14.3	34.0	**0.040**

*PV: Pemphigus vulgaris

USA). For analyses of sequencing data, SeqScape® software v2.5 (Applied Biosystems, USA) was used (21).

Statistical Analysis

The SPSS 13.0 package program was used for statistical analyses. Constant variables in the data set were expressed as mean and Standard Deviation, while categorical variables were expressed as frequency and percentage. The chi-square test was used in group comparisons of categorical variables. The independent samples test was used in two-group comparison of normally distributed constant variables, and the Mann-Whitney U test was used for non-normally distributed constant variables. Significance was defined as $P<0.05$.

RESULTS

Forty-nine Turkish patients with PV and 50 healthy control subjects were enrolled in the study.

We determined the HLA-E genotypes and their frequencies in the patients with PV and control populations. The HLA-E genotypes were HLA-E*0101/*0101, HLA-E*0101/*0103X (*0101/*010301, *0101/*010302), HLA-E*0103X/*0103X (*010301/*010301, *010301/*010302, *010302/*10302), and HLA-E*0101/*0106.

We compared the frequencies of these HLA-E genotypes between the patients and control subjects. We found that patients and control subjects were more likely to type as HLA-E*0101/0103X heterozygous genotype (65.3 % of patients vs. 46.0% of controls; $P=0.084$). Patients with PV were significantly less likely to type as HLA-E*0103X/0103X homozygous genotype than control subjects (14.3% of patients vs. 34.0% of controls; $P=0.040$) (Table 1). For HLA-E*0101/0101 homozygous genotype (18.4% of patients vs. 20.0% of controls; $P=1.000$), the difference was not statistically significant. There was only one patient with HLA-E*0101/0106 heterozygous genotype, while no control subjects had this genotype.

Regarding sex associations, we found that men in the patient group were less likely to type as HLA-E*0103X/*0103X homozygous genotype than men in the control group (3.7% of male patients vs. 40.7% male controls; $P=0.003$) while women in both groups did not show a significant difference in terms of this or any other genotype. A statistically significant difference for male subjects was also found in the comparison of the frequencies of HLA-E*0101/0103X heterozygous genotype (81.5% of male patients vs. 48.1% of male controls; $P=0.023$) (Table 1).

Regarding clinical types of PV, 38 of 49 (77.5%) patients had mucocutaneous PV, while 10 of 49 (20.4%) patients only had mucosal disease. We compared the frequencies of HLA-E genotypes between the patients and control subjects according to the clinical types of the disease. We found that patients with mucosal PV were more likely to type as HLA-E*0101/0101 homozygous genotype than patients with mucocutaneous PV (50% vs. 7.9%; $P=0.006$). Patients with mucocutaneous PV were more likely to type as HLA-E*0101/0103X heterozygous genotype than patients with mucosal PV (78.9% vs. 20.0%; $P=0.001$). For the HLA-E*0103X/0103X homozygous genotype (13.2% vs. 20.0%; $P=0.625$), the difference was not statistically significant (Table 2).

DISCUSSION

In the present study, we found an association between HLA-E and PV. We found that the HLA-E*0103X/0103X homozygous genotype was significantly less frequent in patients with PV, indicating that carrying this homozygous genotype might be a protective factor for PV. We also found that the HLA-E*0101/0103X heterozygous genotype was significantly more frequent in men with PV, indicating that carrying this genotype enhanced the risk of PV in men. In addition, the latter genotype was also significantly more frequent in patients with mucocutaneous PV.

While PV is a rare autoimmune disease, a large number of studies in different populations have been published on the genetic susceptibility to it. They mostly showed that HLA class II antigens were more prevalent in patients with PV than in the general population (5-7,10,13,14).

Table 2. The comparison of the frequencies of the human leukocyte antigen (HLA)-E genotypes according to the clinical types of pemphigus vulgaris

	Mucocutaneous PV (n=38)	Mucosal PV (n=10)	*P*
HLA-E*0101/0101: n (%)	3 (7.9)	5 (50)	**0.006**
HLA-E*0101/0103X: n (%)	30 (78.9)	2 (20)	**0.001**
HLA-E*0103X/0103X: n (%)	5 (13.2)	2 (20)	0.625
HLA-E*0101/0106: n (%)	-	1 (10)	

*PV: Pemphigus vulgaris

There is only one study from North America on HLA-E polymorphisms and PV. Bhanusali *et al.* reported that the homozygous genotype HLA-E*0103X/0103X was associated with susceptibility to PV in a cohort including Caucasian and Ashkenazi Jewish patients (17). In contrast to this report, our study found that patients with PV were significantly less likely to type as the homozygous genotype HLA-E*0103X/0103X (14.3% of patients vs. 34.0% of controls; *P*=0.040), indicating that carrying this genotype might be a protective factor for PV in the Turkish population. The following factors could be speculated as the causes of this discrepancy: First of all, distribution of HLA-E alleles is different between the ethnic groups in these studies, and the subtle genetic difference might make it possible that genetic factors involved in the pathogenesis of PV vary in these populations. Therefore, the putative HLA alleles involved in the pathogenesis of PV in Caucasian and Ashkenazi Jewish may be distinct from the HLA alleles in the Turkish population. Secondly, the interaction between HLA alleles and different infectious agents or environmental allergen across geographical regions might cause this discrepancy. Finally, the limited size of both studies might be another cause of this inconsistency.

In our study, we also found that male patients with PV were significantly more likely to type as HLA-E*0101/0103X heterozygous genotype than men in the control group (81.5% of male patients vs. 48.1 % of male controls; *P*=0.023). This result indicates that carrying this genotype in men increases the risk of PV in the Turkish population. However, in Bhanusali *et al.*, the difference between male subjects in those groups was not significant in terms of this genotype (17).

To the best of our knowledge, our study is the second report on the association of HLA-E polymorphism and PV. Our results do not support the findings of the first study, published by Bhanusali *et al.* These contrasting results may indicate how affected different races and ethnicities are in the HLA-E gene polymorphisms. Moreover, the heterozygous genotype HLA-E*0101/*0106 was determined to be a different HLA-E genotype in our patient group; it was however not found in the former study. This finding shows the diversity among the patient group of our study and also supports the possibility of variations in HLA-E gene polymorphisms.

An increasing number of studies have shown that HLA-E polymorphisms may play an integral role in various autoimmune and inflammatory diseases, including rheumatoid arthritis, type 1 diabetes mellitus, Behcet's disease, multiple sclerosis, celiac disease, and psoriasis (22-7).

HLA-E is the least polymorphic molecule of all class I HLA genes and is characterized by a conserved peptide-binding groove (28,29). The functional roles of these polymorphisms reported in PV and other autoimmune diseases remain unclear. The HLA-E molecule can be recognized by a subset of HLA-restricted regulatory $CD8^+$ T-cells in a T-cell receptor (TCR)-dependent manner; this signaling pathway has been implicated in the pathogenesis of autoimmune diseases (30). Experiments with a murine model of multiple sclerosis (experimental allergic encephalomyelitis; EAE) revealed that engagement of the TCR on regulatory $CD8^+$ T cells by Qa-1 (HLA E in humans)-peptide complexes triggers suppressive activity of these cells towards potentially autoreactive $CD4^+$ T clones, while impairment of this interaction was associated with increased susceptibility to the development of EAE (31). Additionally, *in vivo* studies in patients with type 1 diabetes mellitus confirmed the essential role of the HLA-E restricted $CD8^+$ T-cell-mediated pathway in the maintenance of peripheral self-tolerance (32). Regarding this recognition pathway, it was proposed that the HLA-E*0101 and HLA-E*0103 alleles might differ in their capacity to interact with TCR receptors on regulatory T-cells (17).

Furthermore, NK cells have been shown to be involved in the regulation of autoreactive $CD4^+$ T-cells via the Qa-1-NKG2A inhibitory pathway in experimental murine models of rheumatoid arthritis and multiple sclerosis (33,34). Interruption of the interaction between Qa-1 and CD94/NKG2A through anti-NKG2A antibody administration or genetic disruption has been shown to result in up-regulated activity of NK cells against pathogenic $CD4^+$ T-cells and associated with inhibition of the disease development. The results of these studies suggest that a presence of the Qa-1 molecule on self-reactive $CD4^+$ T-cells confers protection from lysis mediated by $NKG2A^+$ NK cells. In this context, Iwaszko *et al.* hypothesized that the HLA-E polymorphism, resulting in different HLA-E surface expression on the potentially pathogenic autoreactive T-cells, might determine their susceptibility to lysis mediated by $NKG2A^+$ NK cells. According to this scenario, highly expressed HLA-E*0103 molecules may be responsible for increased transmission of inhibitory signals via CD94/NKG2A receptors, leading to decreased ability of NK cells to regulate autoreactive T-cells (27).

In our study, we found that the HLA-E*0101/0103X heterozygous genotype was significantly more frequent in men with PV, indicating that carrying this genotype increased the risk of PV in men. This genotype was also markedly more frequent in the patient group (65.3% of patients vs. 46.0% of controls;

P= 0.084). However the difference was not statistically significant, probably due to low sample size. Despite the relatively low sample size of our study, it is unlikely that the nearly twofold increase in HLA-E*0101/0103X in male patients versus male controls could be attributed to a statistical error. Genetic differences between men and women have been implicated in various sex-specific patterns of susceptibility to autoimmune disorders (35). Regarding sex-associated risks, while pemphigus is generally a disease which is slightly more common in women, our findings may be considered as a valid result for men. Nevertheless, larger multicenter studies in the Turkish population may confirm this sex-specific association.

To the best of our knowledge, there have been no published reports on the association between the clinical types of PV and HLA-E polymorphism. Our first results investigating this relationship revealed that the HLA-E*0101/0103X heterozygous genotype was also significantly more frequent in patients with mucocutaneous PV, clearly the most frequent type of PV in general. This is also consistent with the main result of our study, indicating this genotype might play a role in the susceptibility to PV.

Our study has some limitations: Firstly, the sample size of our study was low; however, several published studies on PV and HLA associations have similar sample size. Secondly, this study is only a single-center case-control study on a Turkish population, and further studies will be required to confirm the implication of HLA-E polymorphisms in the pathogenesis of PV.

CONCLUSION

The results of our study indicate possible involvement of HLA-E in the genetic background of PV in the Turkish population of Caucasian origin. Carrying the HLA-E*0101/0103X genotype may increase the risk of PV in male patients. Further studies will be required to confirm the role of these HLA-E polymorphisms in the pathogenesis of PV.

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