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Association of class II human leukocyte antigen (HLA) alleles with pulmonary sarcoidosis

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Abstract. *Background and objectives:* Sarcoidosis is a systemic inflammatory disease of unknown etiology that involves any part of the body, mainly the lungs and thoracic lymph nodes. The clinical presentation is heterogeneous based on the degree and extent of organ involvement. The existence of variable clinical presentations and treatment responses suggest an important role of genetic predisposition. In genetic studies, sarcoidosis was found to be associated with several genes, but the strongest link was with HLA region. The aim of this study was to investigate the association of HLA class II alleles with the extent and course of disease in Turkish patients with sarcoidosis. *Methods:* The study included 103 patients with sarcoidosis and 100 unrelated healthy controls. HLA-DRB1 and HLA-DQB1 typing was performed by using Polymerase Chain Reaction-Sequence Specific Priming (PCR-SSP) method at low resolution level. *Results:* HLA-DRB1* and -DQB1* analysis revealed that while the frequency of HLA-DRB1*01 was significantly higher in the control group, HLA-DRB1*13 and -DQB1*06 alleles were more frequent in the sarcoidosis patients. When the patients were grouped based on clinical outcome as remitters and non-remitters, HLA-DRB1*10 allele was only detected in the remitters, whereas the frequency of HLA-DQB1*06 allele was significantly higher in non-remitters. *Conclusions:* This study supported the association of HLA alleles with sarcoidosis. In a considerably high number of patients with Turkish origin, the frequency of HLA-DRB1*13, -DRB1*10 and HLA-DQB1*06 alleles was significantly associated with increased risk and clinical outcome. *(Sarcoidosis Vasc Diffuse Lung Dis 2018; 35: 143-149)*

Key words: HLA, genotyping, pulmonary sarcoidosis, interstitial lung disease

Introduction

Sarcoidosis is an inflammatory disease that can affect any organ in the body with a predominance of pulmonary system and mediastinal lymph nodes(1). Despite extensive research regarding the etiology of the disease, the exact factors underlying the aberrant immune response in sarcoidosis is not well-established. It has been acknowledged that in sarcoidosis immunopathology antigen presenting cells (APCs) which interact with T lymphocytes induce the differentiation of type 1 helper T (Th1) cells and lead to accumulation of many other immune cells and mediate the formation of non-caseating granulomas (2). These granulomas either resolve or lead to fibrosis and dysfunction of the organ (3).

The class II major histocompatibility complex (MHC) molecules encoded by human leukocyte an-

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tigens (HLA) are specifically found on the APCs, which play an important role in antigen presentation. HLA loci is the most variable region in the human genome and is responsible for the heterogeneity of individuals' immune response capacity. Therefore, it has been shown by different studies from various countries that HLA alleles are closely related to not only the risk of developing sarcoidosis, but also the type of clinical presentation and outcome (4, 5). Especially in the Scandinavian countries, where the incidence of sarcoidosis is high and the importance of genetics and multifactorial nature of this disease have been emphasized (6, 7).

Similar studies were also performed in Turkey, a country with an incidence rate expected to be around 4/100.000 (8). However three previous studies reported different HLA incidences probably due to low number of patients, usage of old techniques in HLA typing, and only regional involvement of patients not representing whole country (9-11). While the first two studies demonstrated only the frequency of alleles, third study showed a relationship between HLA-DRB1*11 and extra-pulmonary sarcoidosis (11). None of these three studies investigated the association of HLA alleles with clinical outcome.

The aim of this study is to determine the frequency of HLA-DRB1 and HLA-DQB1 alleles in Turkish sarcoidosis patients. A considerably high number of patients from various regions of the country was enrolled and a possible relationship between the HLA alleles and clinical outcome was observed.

Material and Methods

Patients

This study included 103 patients with sarcoidosis and 100 unrelated healthy controls. The patient group contained only Turkish descendants and no one from the minority populations was included in the study. The control group was matched with sarcoidosis patients based on gender, age and geographical region. Of the patients with sarcoidosis, 49 were newly diagnosed during the study, whereas the remaining 54 were follow-up. All clinical parameters of the patients were followed up at least for a year in the Department of Chest Diseases at Hacettepe University Hospital.

The diagnosis of sarcoidosis was confirmed according to the ATS/ERS/WASOG statement and only patients belonging to 'highly probable' and 'probable' category were included (1, 12). All the patients had a clinical and radiological presentation compatible with sarcoidosis and 98 patients had a biopsy demonstrating non-caseating granulomas in at least one involved organ. The most commonly used diagnostic procedures were conventional transbronchial needle aspiration (n=52, 53.6%), biopsy from involved extra-pulmonary organs (n=22, 21.4%) and mediastinoscopy (n=13, 13.4%). The patients who didn't have a biopsy presented either with a typical presentation of Löfgren's syndrome or had typical clinical features and/or magnetic resonance imaging (MRI) findings of brain or eye involvement. This study was approved by the Clinical Research Ethics Committee of Hacettepe University (No: 16969557-924) and all the patients read and signed the informed consents before enrollment.

Available study forms were duly filled in for each patient. A detailed medical history was taken from each patient and a thorough physical examination was performed. Laboratory analysis including complete blood count, liver and renal function tests, angiotension converting enzyme (ACE) level, calcium level in 24 hour collected urine; pulmonary function tests including spirometry and diffusion capacity for carbon monoxide; radiological investigations including a posterior-anterior chest x-ray and computed tomography of thorax if necessary; electrocardiogram and echocardiography were performed. The patients were consulted to cardiology, ophthalmology, dermatology or neurology in case of suspicion of involvement.

HLA typing

A total of 3 mL venous blood was collected from each patient and DNA isolation was performed (QIAamp DNA Blood mini kit, QIAGEN, USA) according to the manufacturer's instructions. The HLA typing was done by using the Sequence Specific Priming (SSP) method. For each patient an AllSet^{+TM} Gold SSP HLA-DRDQ Low Resolution (Invitrogen) kit was used. Briefly primer oligonucleotides specific for HLA alleles and internal control primers were used in a polymerase chain reactions (PCR). PCR products were run in %2 agarose gel

electrophoresis and then visualized under UV light. The analysis of allele distribution for HLA-DRB1 and HLA-DQ1 loci was performed in accordance with the guideline provided by the manufacturer.

Statistical Analysis

SPSS for windows release 22.0 package program was used to carry out the statistical analysis. The descriptive statistics were given as mean ± standard deviation for variables with a normal distribution while median values (minimum-maximum) were used for variables that were not normally distributed, and number of cases (%) for nominal variables. The HLA allele frequency for both patient and control groups were compared by *Chi-square* or *Fisher's exact test*. *Student's t-test* and *Mann Whitney U* tests were used for the comparison of other clinical parameters. Odds ratios and confidence intervals (95%CI) were calculated. Any result with a 'p' value less than 0.05 were considered to be statistically significant.

Results

Patient characteristics

The study included 103 patients (76 females, 27 males) with a mean age of 42.5±12.9 years (Range: 16-71) coming from 43 different cities of Turkey. The female to male ratio was 2.8. While the mean age of females was 44.6±13.5 years with a double peak distribution at 30's and 50's on age graphic, the mean age of males was 36.4±8.9 years with a single peak at 30's (data not shown). The characteristics of patients are summarized in Table 1. Most of the patients (86.4%) were non-smokers or ex-smokers. Ninety five patients (92.2%) had pulmonary system involvement including 6 with a clinical presentation of Löfgren's syndrome. Extrapulmonary organ involvement was present in 57 (55.3%) patients. Patients were mainly (79.6%) symptomatic on admission. Patients presented with mostly respiratory (64.1%) and constitutional symptoms (55.3%). Most of the patients had radiologically stage 1 and 2 pulmonary disease based on posterior-anterior chest x-rays. The pulmonary function test results at initial diagnosis were supplied in 87 patients and they were normal in 38 (43.7%), restrictive in 20 (23%), obstructive in 23 (26.4%) and mixed pattern in 6 (6.9%) patients. The laboratory analysis showed lymphopenia (absolute lymphocyte count <1200) in 26 patients (25.2%), anemia (hemoglobin level less <11 mg/dL) and increased liver enzymes (higher than laboratory reference upper limit) in 16 patients (15.5%), increased ACE levels (>52

Table 1. Patients' clinical characteristics

Patient characteristics	Frequency (%)
Gender	
Male	27 (26.2)
Female	76 (73.8)
Age	
<40	48 (46.6)
>40	55 (53.4)
Smoking status	
Smoker	14 (13.6)
Non-smoker	54 (52.4)
Ex-smoker	35 (34.0)
Symptoms	
No symptoms	21 (20.4)
Respiratory symptoms	66 (64.1)
Constitutional symptoms	57 (55.3)
Other symptoms	31 (30.1)
Radiologic stage	
0	8 (7.8)
1	32 (31.1)
2	58 (56.3)
3	2 (1.9)
4	3 (2.9)
Pulmonary function tests	
Normal	38 (43.7)
Obstructive	23 (26.4)
Restrictive	20 (23.0)
Mixed	6 (6.9)
Not present	16 (15.6)
Laboratory results	
Lymphopenia	26 (25.2)
Anemia	16 (15.5)
Elevated liver enzymes	16 (15.5)
Elevated ACE levels	41 (45.6)
Hypercalciuria	18 (17.5)
Organ involvement (no.)	
1	52 (50.5)
2	33 (32)
3	13 (12.6)
≥4	5 (4.9)
Extra-pulmonary involvement	57 (55.3)
Familial sarcoidosis	6 (6.06)

U/L) in 41 patients (45.6%) and hypercalciuria (Ca level >300 mg/24 hour urine specimen) in 18 patients (17.5%) in initial presentation. The incidence of familial sarcoidosis was calculated as 6.06%.

HLA-DRB1* and -DQB1* genotyping in both patient and control groups revealed a total of 18 different alleles (Table 2). When compared to the patients the HLA-DRB1*01 allele frequency was significantly higher in the control group (OR: 0.327, 95%CI (0.142-0.753, p=0.011). HLA-DRB1*13 and HLA-DQB1*06 alleles were significantly more frequent in sarcoidosis patients (OR: 2.599, 95% CI (1.520-4.442), p=0.001 and OR:1.868, 95% CI (1.157-3.015), p=0.01) as shown in Table 2.

Clinical outcome

The clinical outcome was evaluated in 97 patients who had sufficient data. Six patients were excluded due to absence of final visit evaluation or newly diagnosed patient without a follow-up period of at least 6 months. There were 37 remitters (21 spontaneous remissions, 16 remissions with systemic steroid treatment) and 60 non-remitters (6 patients with progressive disease, 32 patients with stable disease, 22 patients with progressive disease). The comparison of clinical characteristics of patients at the time of diagnosis between remitters and non-remitters revealed that while forced expiratory volume 1 (FEV1) and forced vital capacity (FVC) values were better in remitters, ACE levels were significantly higher in non-remitters (Table 3). The number of organ involvement and radiological stage were lower in the remitter group, but this difference was not statistically significant. In order to investigate if the presence of an HLA allele can associate with the clinical outcomes from the time of diagnosis, we compared allele frequency between remitters and non-remitters. The HLA-DRB1*10 allele was only detected in the remitters (OR: 1.072, 95%CI (1.009-1.140), p=0.007) (Table 4) who were categorized to have a better prognosis. On the other hand the HLA-DQB1*06 allele frequency was significantly higher in non-remitters (OR: 0.45, 95%CI (0.225-0.899), p=0.034) (Table 4). No difference was

Table 3. Comparison of clinical parameters between remitters and non-remitters

Clinical parameters	Remitters (n=37) f (%)	Non-remitters (n=60) f (%)	p
Number of involved organs	1 (1-4)	2 (1-6)	0.081
Radiologic stage	2 (1-2)	2 (1-4)	0.160
FEV1 (%)	**89.24±13.72**	**79.42±18.45**	**0.014**
FVC (%)	**91.87±15.57**	**83.91±18.08**	**0.042**
FEV1/FVC	83.51±8.05	79.72±9.1	0.051
DLCO (%)	81.12±17.65	84.02±29.48	0.667
DLCO/VA	97.50±15.49	107.45±23.07	0.057
Lymphopenia	7 (18.9%)	19 (32.2%)	0.234
High ACE level	**7 (21.9%)**	**29 (54.7%)**	**0.006**

Table 2. HLA-DRB1* and HLA-DQB1* results of patient and control groups

HLA DRB1* alleles	Patient group (n=206) f (%)	Control group (n=200) f (%)	p	OR (CI)
DRB1*01	**8 (3.9)**	**22 (11.0)**	**0.011**	**0.327 (0.142-0.753)**
DRB1*03	12 (5.9)	14 (7.0)	0.779	0.822 (0.370-1.823)
DRB1*04	20 (9.8)	26 (13.0)	0.296	0.720 (0.388-1.336)
DRB1*07	13 (6.3)	24 (12.0)	0.069	0.494 (0.244-1.00)
DRB1*08	4 (1.9)	2 (1.0)	0.708	1.960 (0.355-10.824)
DRB1*09	1 (0.5)	1 (0.5)	1.00	0.971 (0.060-15.626)
DRB1*10	5 (2.4)	6 (3)	0.96	0.804 (0.242-2.679)
DRB1*11	39 (18.9)	44 (22)	0.443	0.828 (0.511-1.342)
DRB1*12	6 (2.9)	2 (1)	0.303	2.970 (0.592-14.893)
DRB1*13	**52 (25.2)**	**23 (11.5)**	**0.001**	**2.599 (1.520-4.442)**
DRB1*14	17 (8.3)	13 (6.5)	0.628	1.294 (0.611-2.739)
DRB1*15	25 (12.1)	16 (8.0)	0.223	1.588 (0.821-3.074)
DRB1*16	4 (1.9)	7 (3.5)	0.509	0.546 (0.157-1.895)
DQB1*02	23 (11.2)	29 (14.5)	0.392	0.741 (0.413-1.331)
DQB1*03	84 (40.8)	86 (43)	0.650	0.913 (0.615-1.354)
DQB1*04	4 (1.9)	3 (1.5)	1.000	1.300 (0.287-5.855)
DQB1*05	38 (18.4)	48 (24.0)	0.171	0.716 (0.444-1.156)
DQB1*06	**57 (27.7)**	**34 (17.0)**	**0.010**	**1.868 (1.157-3.015)**

Table 4. HLA-DRB1* and HLA-DQB1* allele expression in remitters and non-remitters

HLA DRB1* alleles	Remitters (n=74) f (%)	Non-remitters (n=120) f (%)	p	OR (CI)
DRB1*01	2 (2.7)	5 (4.2)	0.893	0.639 (0.121-3.381)
DRB1*03	4 (5.4)	8 (6.7)	0.962	0.800 (0.232-2.756)
DRB1*04	8 (10.8)	8 (6.7)	0.453	1.697 (0.608-4.735)
DRB1*07	7 (9.5)	6 (5.0)	0.362	1.985 (0.640-6.154)
DRB1*08	1 (1.4)	2 (1.7)	1.00	0.808 (0.072-9.072)
DRB1*09	0 (0)	1 (0.8)	1.00	0.992 (0.976-1.008)
DRB1*10	**5 (6.7)**	**0 (0)**	**0.007**	**1.072 (1.009-1.140)**
DRB1*11	15 (20.3)	21 (17.5)	0.770	1.199 (0.574-2.504)
DRB1*12	1 (1.4)	4 (3.3)	0.704	0.397 (0.044-3.624)
DRB1*13	18 (24.3)	33 (27.5)	0.749	0.847 (0.436-1.648)
DRB1*14	4 (5.4)	13 (10.8)	0.300	0.470 (0.147-1.501)
DRB1*15	6 (8.1)	18 (15)	0.233	0.500 (0.189-1.324)
DRB1*16	3 (4.0)	1 (0.8)	0.311	5.028 (0.513-49.266)
DQB1*02	10 (13.5)	13 (10.8)	0.740	1.286 (0.533-3.103)
DQB1*03	33 (44.6)	42 (35.0)	0.183	1.495 (0.827-2.703)
DQB1*04	1 (1.4)	3 (2.5)	0.979	0.534 (0.055-5.233)
DQB1*05	16 (21.6)	21 (17.5)	0.602	1.300 (0.629-2.690)
DQB1*06	**14 (18.9)**	**41 (34.2)**	**0.034**	**0.450 (0.225-0.899)**

found when HLA allele frequencies were distributed amongst the patients with isolated pulmonary and extra-pulmonary involvement (p>0.05).

Discussion

Sarcoidosis is a disease of unknown etiology with a high variety of clinical phenotypes and outcomes that has been shown to be related with the individual's genetic background. The strongest association is with the HLA region (4). In this study, we demonstrated that there is a strong association of the HLA-DRB1*13, -DRB1*10 and HLA-DQB1*06 alleles and increased risk for sarcoidosis in a Turkish cohort.

Sarcoidosis can be classified as a rare disease in Turkey with an incidence rate estimated to be 4/100.000 (8). This rarity might have been a challenge for including higher number of patients in the previous studies (9-11). 103 patients included in this study came from 43 different cities of Turkey and showed a heterogeneous distribution thus increasing the probability of being a representative group. Here the female to male ratio was 2.8 and the mean age of diagnosis for women was higher than men, which is attributed to the 'double peak' theory in Japanese and European studies showing that sarcoidosis can be common in 30's and 50's in women (1, 13).

Extra-pulmonary involvement of sarcoidosis varies from 16.6 to 39% (14, 15). It was previously reported as 40.6-42.9% in Turkish patients (8, 16), however our study demonstrated a higher ratio for extrapulmonary involvement. Non-specific findings together with the absence of symptoms is the major drawback for the diagnosis of extra-pulmonary involvement. In addition sarcoidosis is not only sporadic but also familial. The incidence of familial sarcoidosis varies from 2.8 to 18% in various studies with the highest ratio in Non-Hispanic Whites (17, 18). In contrast to previous Turkish data, in which the ratio of familial cases was less than 1% (8), according to the present findings the ratio of familial sarcoidosis was higher (6%). Genetic association studies especially on the highly polymorphic genomic regions are effected by small sample sizes, variations in geographical areas and also the technique used for analysis.

There are many different HLA alleles reported from various countries to be associated with higher or lower risk for the disease development and reproducibility has been of increased concern for them, too (19, 20). For example, HLA-DRB1*13 allele has been shown to be associated with an increased risk of non-Löfgren's disease in Czech population, whereas HLA-DQB1*06 allele was associated with non-Löfgren's disease in White Dutch and progressive pulmonary disease in African Americans (21, 22).

In contrary to these findings, another study showed a protective role of HLA-DQB1*0603 in US males (23). Another remarkable finding of this study was significantly higher expression of HLA-DRB1*01 allele in the control group (p=0.01) suggesting a protective role against the disease. This finding which was not reported in previous Turkish studies was compatible with many international studies showing a decreased risk for disease in White Swedish, UK, Dutch, Japanese and Finnish populations (6, 22, 24, 25). In a previous Turkish Study (11), HLA-DRB1*11 allele was suggested to be related with extra-pulmonary sarcoidosis in Turkish patients, but in this study we could not demonstrate such a relationship.

The clinical presentation of sarcoidosis is heterogeneous based on the degree and extent of organ involvement. While some patients recover spontaneously or with medical therapy, some progress insidiously and present with chronic fibrotic disease. At the time of initial diagnosis, it is difficult to predict the clinical outcome and prognosis (26). Foreseeing the disease prognosis from the very beginning might help to organize a treatment plan, estimate therapy duration and arrange follow-up schedule. Unfortunately there is not 'a magical' criteria or laboratory test result that can do this, but a strong effort was spent in finding a relationship between HLA alleles and specific clinical outcomes. For example in Scandinavian countries HLA-DRB1*03 allele is strongly associated with acute onset, Löfgren's syndrome and good prognosis (6); HLA-DRB1*14 and -DRB1*15 are shown to be related with prolonged disease (19); HLA-DRB1*04 associated with especially ophthalmic involvement and Heerfordt's syndrome (24). The association of HLA-DQB1*06 allele with non-remitting disease may indicate a worse prognosis and HLA-DRB1*10 allele expression with remitting disease may indicate a better prognosis.

There are some limitations of this study. Firstly, the number of patients included in this study is still low and these are the results of a single medical center. The collaboration of different medical centers dealing with sarcoidosis located in different geographical areas might be a solution for random sampling of a representative group. Secondly, the follow-up time of some patients was not long enough to decide for clinical outcome. It is known that sarcoidosis can undergo remission in 1-2 years, but sometimes this period can be up to 5 years. In our study mean follow-up time of the patients was 3.78±0.9 years and at least 6 months of follow-up was accepted as adequate for newly diagnosed patients in order to decide about the prognosis.

Conclusions

This study showed that the HLA alleles and sarcoidosis risk for Turkish patients might be different than previously reported and for the first time the association of such alleles and clinical outcome was assessed. There was a strong association of HLA-DRB1*13, -DRB1*10 and HLA-DQB1*06 alleles and increased risk of sarcoidosis in a Turkish cohort. Additionally there was an association between HLA-DQB1*06 allele expression with non-remitting disease indicating poor prognosis and HLA-DRB1*10 allele expression with remitting disease indicating a better prognosis. Based on these findings we suggest that the analysis of HLA alleles at the time of diagnosis might predict the clinical outcome and help designation of treatment strategy.

Author contributions: DE has contributed as a guarantor author in designing, planning of the research, active contribution in patient follow-up and experimental work, statistical analysis and commenting of the results, writing of the manuscript.; FO has contributed in HLA typing of patients and control group and commented the results; DK has contributed in patient follow-up and diagnostic work, and editing the manuscript; SO has contributed in pathological examination of the biopsy specimens; SE has contributed in both design and comment of the results. All the authors have approved the final version of the manuscript.

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