SARCOIDOSIS VASCULITIS AND DIFFUSE LUNG DISEASES 2014; 31; 154-162

Could HLA-DR B1*11 allele be a clue for predicting extra-pulmonary sarcoidosis?

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Abstract. *Background:* Several HLA-DR alleles have been described as a potential risk factor for sarcoidosis between distinct ethnic groups however the relationship between HLA-DR alleles and extra-pulmonary sarcoidosis (EPS) is still scarce. *Objectives:* The aim of this prospective study is to investigate the relationship between extra-pulmonary involvement and HLA-DR genetic analysis in Turkish patients with sarcoidosis. *Methods:* In this study, we HLA-typed sarcoidosis patients with and without extra-pulmonary involvement, and compared with healthy control subjects. The presence of EPS was evaluated with previously defined standard criteria (ACCESS) and only patients with definite and probable involvement were accepted as positive. Sequence Specific Oligonucletide Probes method was used for typing of HLA-DRB1 alleles from DNA samples in both groups. *Results:* The frequency of HLA DRB1*15 allele was more frequent in patients with sarcoidosis than controls (% 20.4 vs % 9.6)(pcorr=0.017). According to multivariate analysis (MVA), the presence of HLA DRB1*15 was indicated as an independent risk factor for sarcoidosis (OR:2.37; 95% CI: 1.31-4.30, p=0.004). Extra-pulmonary involvement was present in 39 patients (42.9 %). When the patients with and without extra-pulmonary involvement compared, HLADRB1*11 allele was significantly higher in patients without extra-pulmonary sarcoidosis which may be concluded as a protective allele for systemic involvement (%30.8 vs. %15.4)(p<0.05). This result was also confirmed with the MVA (OR:0.35, %95 CI:0.15-0.84, p=0.018). *Conclusions:* We demonstrated a strong positive link between the haplotype HLA DRB1*15 and sarcoidosis in a Turkish Caucasian population and a potential protective effect of HLA DRB1*11 from extra-pulmonary involvement of disease. *(Sarcoidosis Vasc Diffuse Lung Dis 2014; 31: 154-162)*

Key words: Sarcoidosis, HLA-DR alleles, extra-pulmonary involvement

Introduction

Sarcoidosis is a complex systemic inflammatory disorder of unknown etiology characterized pathologically by non caseating granulomas that can manifest in virtually any organ system (1). Extra-pulmonary sarcoidosis represents a significant challenge both in its diagnosis and management. It may also be a major cause of morbidity and mortality, as well (2). Racial and gender differences are reported in patients with extra-pulmonary sarcoidosis and the clinical course is unpredictable (2-4).

The exact immunopathogenesis of sarcoidosis still remains an enigma despite there has been tremendous evolution in the past decade. The hypothesis is that; some antigen(s) enter the host and are phagocytosed by antigen presenting cells

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(APCs), mainly macrophages or dendritic cells (5). These APCs process the antigen and subsequently present it to CD4 T-lymphocytes class via human leukocyte antigen (HLA) class II molecules (6-8). Therefore, the key event in the pathogenesis of sarcoidosis seems to involve the interaction between the antigen, HLA Class II molecules and T-cell receptors (8).

The research of the genes which potentially take part in the pathogenesis of sarcoidosis has focused on the HLA genes which is a highly variable genetic system constituted by six main polymorphic loci, A, B, C, DRB1, DQB1, and DPB1, located on the short arm of chromosome 6 (9). The first genetic linkage analysis on sarcoidosis was performed with Schurmann et al. which showed a significant linkage for the chromosome 6p12 to 22, including the HLA region (10). In two previous studies from our country with limited number of patients; increased frequency of HLA A2, A24, A26, A62, A69, 12, B22, B38, B49, DR4, DR14 and decreased frequency of A24, A26, B62, B7, DR7 were implicated (11, 12). In a multicenter epidemiological trial in the United States (A Case Controlled Etiologic Study of Sarcoidosis)(ACCESS), HLA-DRB1*1101 allele was documented as risk factors for disease (13). Subsequently, genetic associations with specific HLA alleles including HLA-DRB1*03, HLA-DRB1*11, HLA-DRB1*12, HLA-DRB1*14 and HLA-DRB1*15 and sarcoidosis have been confirmed (4, 14, 15). Recently; it has been documented that the phenotype and outcome of sarcoidosis is probably influenced strongly by HLA genes (8). A number of investigations showed associations between HLA-DQ Class II variants and extra-pulmonary involvement. In a Dutch study, HLA-DQ*0602 has been indicated to correlate with small fibre neuropathy in sarcoidosis (16). In Japan, patients with splenomegaly had a significantly higher frequency of HLA-DQB1*0602 (17). As well, Japanese patients with cardiac sarcoidosis has been shown to correlate with HLA-DQB1*0601, an unusual allele among white population (18). However, only a few studies have addressed the issue of associations between HLA-DR alleles and specific clinical manifestations. HLA-DRB1*03 allele were reported to be strongly related with Lofgren Syndrome and also disease resolution according to several reports (14, 19-21). An association between HLA-DRB1*04 and ocular sarcoidosis has been identified (13,22). HLA-DR alleles in other forms of extra-pulmonary involvement have not been elucidated yet. The aim of this prospective study is to investigate HLA-DR genetic analysis in a Turkish cohort with sarcoidosis and explore any relationship between extra-pulmonary involvement.

Methods

Ninety-one consecutive adult patients who were diagnosed at Department of Pulmonary Disease of Cukurova University Faculty of Medicine from January 1993 to March 2013 and followed up until September 2013 were recruited for the study. All participants were Caucasians of Turkish origin and were recruited from the Southern part-Easter Mediterranean region of our country. None were related. The diagnosis of sarcoidosis was confirmed with the presence of clinical symptoms, radiological features compatible with sarcoidosis, and biopsy evidence of noncaseating epitheloid cell granulomas in 92.3 % after exclusion of other known causes of granulomatosis as outlined by the joint statement of the American Thoracic Society (ATS), the European Respiratory Society (ERS), and the World Association of Sarcoidosis and other Granulomatous Disorders (WASOG) (1). In this group of patients, 7 patient also were diagnosed as Lofgren's Syndrome. In the remaining 7.7 %, the diagnosis was based only on Lofgren's syndrome which was defined as bilateral hilar lymphadenopathy, fever, ankle arthralgia and erythema nodosum. None of the patients received steroid therapy at the time of diagnosis. Control group were matched to cases in terms of age, gender, origin (Caucasian) and geographical region of residence.

After obtaining diagnosis, the patients underwent a standard evaluation. The following items were recorded: (i) A detailed medical history (including demographical information, environmental exposure, questioning for the each system for extra-pulmonary involvement, current comorbidities, family history of sarcoidosis, and date of sarcoidosis symptoms onset); concurrently physical examination, (ii) posteroanterior chest X-ray and computed tomography (CT); and hand radiography, (iii) lung function tests including carbon monoxide diffusion capacity (D_{LCO}), (iv) elec-

trocardiogram and echocardiogram, (v) abdomen ultrasonography, (vii) ophthalmologic investigation and (vii) tuberculin skin test, (viii) urinary calcium excretion in 24-hours. In case of suspected central nervous system, heart or intra-abdominal organ involvement; magnetic resonance imaging of the brain, gallium scan of heart, CT scan of abdomen was also performed. Organ involvement was determined in each patient, within an assessment system which was previously defined. The date and site of a confirmatory biopsy was noted. Extra-pulmonary involvement was defined positive if it met the criteria for "definitive" or "probable" involvement according to ACCESS organ assessment instrument. Consequently, the timing, the method of diagnosis and any therapy used were recorded in details.

Venous blood samples were obtained from the patient group for whole blood cell count, serum total biochemistry, liver function studies, serum calcium assay and HLA-DR allele genetic analysis. The laboratory investigation other than HLA typing was performed at the same day. Chest X-ray at the time of diagnosis and follow-up were defined by experienced chest physicians specialized in diffuse lung disease, blinded to genotype, in terms of five "Scadding Stages" (Stage 0 to IV) in accordance with the ATS/ERS/WASOG Statement (1). Most of the patients were followed-up with the same investigator in every three months.

Pulmonary Function Tests

Pulmonary function tests (PFTs) were performed in the stable phase by using a calibrated Jaeger MS Spirometer according to the ERS guidelines (23). None of the patients were receiving oral or inhaled short-acting beta 2 agonists 8 h before testing. Baseline forced expiratory volume (FEV_1) and forced vital capacity (FVC) was measured 3 times and the best of 3 measurements was recorded for the analysis. Total lung capacity was measured using the helium dilution technique (Jaeger MS-PFT Analyser Unit). The transfer factor of the lung for carbon monoxide (T_{LCO}) was measured using the single breath method. The results were presented as the percentages of predicted.

The institutional ethics committee approved the study and written informed consents were obtained from all of the participants.

Typing HLA HLA-DRB1 alleles

DNA from venous blood sample of each subject was extracted by DNA isolation kit (QIAamp DNA blood mini kit, cat no: 51104, QIAGEN Vertriebs GmbH, Vienna, Austria). Typing of HLA-DRB1 alleles from DNA samples were performed by Sequence Specific Oligonucletide Probes (SSOP) method in both groups. Tepnel Lifecodes HLA-DRB (Ref:628759-50, lot no: 10102Y, Connecticut, USA) typing kits were used for polymerase chain reaction and hybridization procedures. This product consists of a mixture of locus-specific oligonucleotide probes coupled to color-coded microspheres (Luminex Corp) and two PCR reactions. To type each sample, PCR was performed and the product was hybridized with the SSO-probe mixture using the manufacturer's protocol. After hybridization, the sample plate was placed in a Luminex instrument for analysis.

Statistical Analysis

For each continuous variable, normality was checked by Kolmogorov Smirnov and Shapiro-Wilk tests and by histograms. Comparisons between groups were applied using the student t test for normally distributed data and Mann Whitney U test was used for the data not normally distributed. The categorical variables between the groups were analyzed by using the Chi square test. Data was expressed as mean±SD, median (min-max) and n (%). A p value <0.05 considered as significant. Statistical analysis was performed using the statistical package SPSS v 19.0.

HLA analysis toll that exist in web page of Los Alamos National Laboratory (http://www.hiv.lanl.gov/content/immunology/hla/) were used for statistical analyses of HLA. For each HLA, the tool computes the 2-sided exact Fisher's p-value, which represents the probability that the observed difference is due to chance. P less than 0.05 was considered significant. To correct for the false discovery rate caused by the calculation of multiple p- values, Storey's q-value is also provided (24). A logistic regression analyses was performed to determine the independent risk factors.

Results

Seventy one female, twenty male a total of ninety-one patients with sarcoidosis with a mean age of 45.1 ± 10.1 at the time of diagnosis and 145 healthy controls with a similar age (48.3 ±14.1) and gender (female/male:113/32) were included for the study. Patient characteristics are summarized at Table 1. The majority of patients were never smokers (68.1 %), of whom 24.2 % were ex-smokers and 7.7 % were current smokers. Any pulmonary symptom was present in 84.6 % including cough with being the most common symptom in 46.2 % which is followed by dyspnea (31.9%), chest pain (13.2%), sputum production (9.9%) Constitutional symptoms including weight loss, fever, fatigue and malaise were present in 31.9 %. None patient had haemoptysis whereas 14 patients were asymptomatic. The duration of symptoms was 6.9 ± 12.2 months and the duration of follow-up was 3.9±3.8 years. There were 31 patients in Stage I, 51 patients in Stage II and 9 patients in Stage III at the time of diagnosis. There were no patients in Stage IV. 30 (32.9%) of patients were given treatment during follow-up while 61 (66.1%) were never required any treatment (not shown on Table 1).

Table 1. Characteristics of the patient group

Characteristics	Patients N=91 (%)	Controls N=145 (%)
Age(Mean±SD) (for patients at disease onset)	45.1 ± 10.1	48.3± 14.1
(Median)(Min-Max)	46 (19-80)	48 (22-78)
Gender		
Females	71 (78)	113 (77.9)
Males	20 (22)	32 (22.1)
Smoking history		
Never smokers	62 (68.1)	
Ex-smokers	22 (24.2)	
Current smokers	7 (8.8)	
Diagnosis with biopsy	84 (92,3)	
Prevalence of Lofgren's Syndrome	14 (15.2)	
Extra-pulmonary involvement	39 (42.9)	
Skin	18 (19.7)	
Eye	7 (7.7)	
Hypercalcemia	6 (6.6)	
Peripheral lymph node	5 (5.5)	
Liver	2 (2.2)	
Spleen	2 (2.2)	
Joint	2 (2.2)	
Neurological	1 (1.1)	
Upper respiratory tract	1 (1.1)	

Extra-pulmonary involvement was present in 39 patients (42.9 %). The rate of extra-pulmonary involvement was comparable both among female and males (40.8 % vs. 50.0 %, respectively) and patients less and more than 40 years old at the time of diagnosis (50.0 % vs. 40.0 %) ($p>0.05$). The most common extra-pulmonary involvement was skin (n=18) followed by Lofgren's syndrome (n=14). According to the clinical criteria of extra-pulmonary involvement, all patients with skin, eye, liver, neurological involvement and hypercalcemia were "definite". Although all patients with peripheral lymph node, spleen and upper respiratory tract involvement were "probable" according to the clinical criteria, biopsy confirmation was performed in all; so they were also accepted as "definite" involvement. One patient with spleen involvement was probable. Although the remaining two patients with joint involvement were "possible"; extra-pulmonary involvement was accepted as positive since both of them had other definite systemic involvement including skin and eye. As a result, in 97.4 % of our patients, extra-pulmonary involvement was definite.

When the patients with and without extra-pulmonary involvement compared, age, gender, smoking history, family history of sarcoidosis, respiratory function tests and the frequency of symptoms were comparable unless constitutional symptoms were more common in patients with extra-pulmonary involvement (Table 2).

Twelve different HLA-DR alleles including HLA-DRB1*01, HLA-DRB1*03, HLA-DRB1*04, HLA-DRB1*07, HLA-DRB1*08, HLA-DRB1*10, HLA-DRB1*11, HLA-DRB1*12, HLA-DRB1*13 HLA-DRB1*14, HLA-DRB1*15, HLA-DRB1*16, were determined in patients with sarcoidosis. In addition to these alleles, HLA-DRB1*09 allele was found only in 1 patient in the control group. The frequency of HLA DRB1*15 allele was more frequent in patients with sarcoidosis than controls which was determined as a risk factor for disease (% 20.4 vs % 9.6)(p_{corr}=0.017) (Table 3). A logistic regression analyses was performed whether determined the HLA DRB1*15 genotype is a risk factor for sarcoidosis compared to healthy controls. Due to the insufficient sample size related to homozygote group of HLA DRB1*15 (n=4), we have reclassified homozygote patients and added them into the heterozygote group (n=33); and

Table 2. Comparison of the patients according to the presence of extrapulmonary involvement

	Extra-pulmonary involvement (+) (n=39)	Extra-pulmonary involvement (-) (n=52)	p
Age (years)	47.2 ± 8.9	49.5 ± 10.7	0.283
Female Sex (n) (%)	29 (74.4 %)	42 (80.8 %)	0.610
Smoking history	11 (28.2 %)	18 (34.6 %)	0.650
Constitutional symptoms	19 (48.7 %)	10 (19.2 %)	0.003
FEV1/FVC	79 ± 8.8	78.8 ± 7.8	0.895
FEV1 (%)	87.2 ± 18.1	85 ± 17.5	0.573
FVC (%)	92.2 ± 14.2	90.7 ± 16.7	0.659
DLCO	76.2 ± 17.2	79.2 ± 16.7	0.419
DLCO/VA	90.7± 19.3	93.3 ± 14.9	0.496

Table 3. Comparison of HLA Class II alleles among patients and controls

HLA DRB1 Alleles	Patients (n=91)(%) Allele:182	Controls (n=145)(%) Allele:290	p-value	p-corrected
DRB1*01	3 (1.7)	15 (5.2)	0.081	
DRB1*03	11 (6.0)	33 (11.4)	0.0725	
DRB1*04	23 (12.6)	41 (14.2)	0.681	
DRB1*07	10 (5.5)	29 (10.0)	0.0887	
DRB1*08	1 (0.5)	4 (1.4)	0.653	
DRB1*09	0 (0)	1 (0.3)	1	
DRB1*10	3 (1.6)	10 (3.5)	0.387	
DRB1*11	44 (24.2)	52 (17.9)	0.126	
DRB1*12	3 (1.7)	3 (1.0)	0.68	
DRB1*13	20 (11.0)	35 (12.1)	0.77	
DRB1*14	15 (8.2)	14 (4.8)	0.168	
DRB1*15	37 (20.4)	28 (9.6)	0.00148	0.017
DRB1*16	12 (6.6)	25 (8.6)	0.485	

compared two groups as having HLA DRB1*15 (n=33) or not (n=58). In this multivariate analysis, the presence of HLA DRB1*15 was indicated as an independent risk factor for sarcoidosis (OR:2.37; 95% CI: 1.31-4.30, p=0.004). When the patients with and without extra-pulmonary involvement compared, HLADRB1*11 allele was present significantly higher in patients without extra-pulmonary sarcoidosis which may be concluded as a protective allele for systemic involvement (%30.8 vs. %15.4) ($p<0.05$) (Table 4). This result was also confirmed with the MVA which was performed at the same method (OR:0.35, %95 CI:0.15-0.84, p=0.018). There was no other relationship between either other systemic involvement or Lofgren's Syndrome and HLA-DR alleles.

Discussion

The relationship between sarcoidosis and several HLA Class II alleles has been well described in different populations. However; a consensus about which HLA allele is more significant in sarcoidosis has not been clarified yet, owing to the varied results from studies of different cohorts, ethnicity and race. In addition, the data which shows the link between different clinical phenotypes and HLA alleles is

Table 4. Comparison of HLA Class II alleles in patients according to the presence of extrapulmonary involvement

HLA Alleles	Extrapulmonary Involvement (+) (n=39) (%) Allele=78	Extrapulmonary Involvement (-) (n=52)(%) Allele=104	p-Value
DRB1*01	1 (1.3)	2 (1.9)	1
DRB1*03	6 (7.7)	5 (4.8)	0.533
DRB1*04	10 (12.8)	13 (12.5)	1
DRB1*07	3 (3.8)	7 (6.7)	0.519
DRB1*08	0 (0)	1 (0.09)	1
DRB1*10	2 (2.6)	1 (0.9)	0.577
DRB1*11	12 (15.4)	32 (30.8)	0.0224
DRB1*12	1 (1.3)	2 (1.9)	1
DRB1*13	11 (14.1)	9 (8.6)	0.338
DRB1*14	8 (10.3)	7 (6.7)	0.424
DRB1*15	19 (24.4)	18 (17.3)	0.268
DRB1*16	5 (6.4)	7 (6.7)	1

scarce. In the present study, we demonstrated a strong positive link between the haplotype HLA DRB1*15 and sarcoidosis in a Turkish cohort. In addition, according to the best our knowledge, this is the first report to describe a potential protective effect of HLA DRB1*11 from extra-pulmonary involvement of disease, as well. However, this is a relatively small study and our result indicating a link between HLA-Class II alleles and extrapulmonary involvement of sarcoidosis needs to be reproduced before strongly commenting on.

Although the aetiology remains a debate, there is substantial evidence supporting a genetic predisposition to sarcoidosis. Monozygotic twins are more prone to develop disease than dizygotic twins (25, 26). As well, familial clustering has been reported in sarcoidosis (27). In the largest trial, A Case Control Etiologic Sarcoidosis Study (ACCESS) including 706 index cases, 706 matched control and information on more than 10,000 first degree and 17,000 second degree relatives revealed a familial relative risk of 4.7. White cases had a markedly higher familial relative risk compared with African American cases (18.0 versus 2.8; p=0.098) (27). Another bridge on this wall; is the wide variation of disease frequency and clinical picture between different geographical area and racial groups (3, 4, 28). African-Americans are affected more frequently than whites, tend to more often have a severe and chronic disease. As well, the localization of extra-pulmonary involvement has been reported to be quite different such as African-Americans are more likely to develop skin, liver, lymph node, and ocular involvement whereas erythema nodosum, which is a commonly observed clinical feature in Europeans is rare in black and Japanese populations (1, 3, 28).

Identifying the potentially sub phenotype specific genetic risk factor may contribute as a future prognostic marker for the clinical course of disease (29, 30). Accordingly, research on sarcoidosis has focused on genetic risk factors in the last decade. Class II human leukocyte antigens (HLAs) are cell surface proteins that has been accused to play a key role in several autoimmune diseases (31). The most significant genetic link with sarcoidosis is documented

within the HLA class II region, where specific alleles have been shown to be related with amplified disease risk. In fact, HLA Class II fits well in the pathogenesis of disease due to its critical role in antigen presentation and immunoregulation. Among the HLA class II genes, HLA DRB1 predominates in the sarcoidosis literature. In the previously published largest HLA-survey, the "A Case Control Etiologic Study of Sarcoidosis" (ACCESS), which includes 474 HLA-typed patients, DRB1*11 and DRB1*15 were documented as risk factors for sarcoidosis (13).

Most of the other investigators confirmed these results (15, 32-35) and some others also showed a link between DRB1*04, DRB1*12, DRB1*14, and DRB1*17 and increased sarcoidosis risk (4, 14, 32, 36). Although ethnical differences exist between several populations, we have also confirmed the previously reported association between HLA DRB1*15 and increased sarcoidosis risk in a group of Turkish sarcoidosis patients. Two previous reports from our country also analyzed HLA antigens in Turkish sarcoidosis patients. In the first report, Celik et al. performed HLA-A, HLA-B, HLA-C and HLA-D typing in 83 patients and 250 healthy controls with a former diagnostic tool (microtoxicity) which revelaled no significant difference between both patients and controls after Bonferroni correction and those with and without extrapulmonary involvement (11). The second study, HLA polymorphism was studied in 64 Turkish patients with biopsy confirmed sarcoidosis and showed higher DR 4 and DR 14 antigens along with lower DR 7 antigens by using the same method. HLA-DR15 was not significant among patients and controls (12). The difference from our results may be due to distinct methods used for HLA typing and/or geographical variability of our country.

Extra-pulmonary manifestations are common and comprise a significant proportion of treatment requirement in sarcoidosis. In the ACCESS trial, exactly half of the patients (368/736) had concomitant extra-pulmonary involvement (3). In a recent prospective multicenter trial from our country, extrapulmonary involvement was reported in 40.6 % of 293 sarcoidosis patients which was nearly comparable with our study group with a rate of 42.9 % (37). However, the rate of involvement of each site was not similar among the two reports, possibly due to the discrete diagnostic criteria and/or follow-up of our patient group.

Although evident correlation has been observed between DRB1 alleles and disease risk, current knowledge; among these alleles and extra-pulmonary involvement is still limited. Several reports indicated a relationship between HLA-DRB1*03 allele and Lofgren Syndrome (14, 19-21, 34). As well, an association between HLA-DRB1*04 and ocular sarcoidosis has been identified (13). In a multicenter study which investigate HLA Class II allele differences between different clinical phenotypes across various ethnic groups revealed HLADRB1*08 as a risk factor for neurosarcoidosis in a Japanese population (4). Our present study showed firstly the protective effect of DRB1*11 from extra-pulmonary involvement. HLA DRB1*11 has been documented to be protective from several other disorders including primary biliary cirrhosis, multiple sclerosis, severe liver damage in chronic hepatitis C virus infection and nasopharyngeal carcinoma (38-41) however no report showed this effect in sarcoidosis, to date. Previously, the possible reason of these protective alleles was concluded with the light of the biochemical properties of these alleles. It has been shown that the two well-known protective allele (HLADRB1*01 and *04) share hydrophobic residues at their only variable position (ie, position 11). The remaining nonprotective alleles (HLADRB1*08, *09, *12, *14, *15 and *17) shared a hydrophilic residue at this position (42). Position 11 which is located within the pocket of the antigen presenting channel of the HLA-DR molecule and this may determine peptide binding characteristics, which in turn determine the efficiency

of antigen presentation. This leads to the hypothesis that the protective alleles initiate a highly efficient immune response which eliminates the sarcoid antigen and results in the manifestation of disease (43). This idea was supported with a Dutch study which showed an association of variants of pocket nine in DQ and pocket 5 in DR with different radiologic stages (44). The potential protective effect of DRB1*11 in extra-pulmonary involvement of sarcoidosis should further be elucidated in larger trials among different ethnic groups.

Several limitations of the present study must be taken into account. First, we have classified patients according to ACCESS extra-pulmonary involvement defining criteria and only dealt with definite and probable extra-pulmonary involvement. Possible involvements were excluded from the analysis. Thus, the potential limitations of this system which were discussed elsewhere may be concluded for this study (45). Second, we did not investigate any specific environmental factors which were beyond the scope of this study. Environmental-genetic interactions have been accused as a potential etiologic factor in sarcoidosis. In fact, in a study using the ACCESS study data, exposures to high humidity and water damage were found to augment the protective effect of the DQB1*02 allele (46). High humidity and sunlight

are also characteristic features of our geographical region; however we could not comment on this theme, at this moment. Third, current data are not adequate to allow us to draw a conclusion regarding the association between HLA alleles and prognosis due to the reason that a significant proportion of our patients have not achieved a sufficient follow-up period. Finally, the results of the present study display only a small sample size of population and should be confirmed before commenting on a clear relationship between HLA-Class II alleles and extrapulmonary involvement of disease.

In conclusion, this study indicated that the categorization of the disease phenotype (e.g., presence or absence of extra-pulmonary involvement) with genotyping of the related gene (HLA DR) may provide a prediction of disease course, treatment requirement or choice in patients with sarcoidosis. Further genetic trials in diverse populations are needed in order to elucidate the potential pathogenic mechanism of disease.

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