# HLA class I and class II frequencies in patients with sarcoidosis from Croatia: role of HLA-B8, −DRB1*0301, and −DQB1*0201 haplotype in clinical variations of the disease

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## Abstract

Sarcoidosis is an immune-mediated, multiorgan, granulomatous disease triggered by a combination of environmental and genetic factors. Numerous studies have reported about an association of human leukocyte antigen (HLA) alleles with sarcoidosis, with variation of alleles in different ethnic groups. Therefore, we investigated 142 Croatian sarcoidosis patients treated at the University Hospital for Lung Diseases 'Jordanovac', Zagreb, Croatia. Diagnosis was based on the presence of typical clinical features, chest X-ray findings and biopsy evidence of granuloma. Patients and control subjects ($n = 190$) were typed for HLA class I antigens by serology, while for HLA class II, they were tested by the polymerase chain reaction-sequence specific primers (PCR-SSP) method. Results indicated that HLA-B8, −DRB1*0301, and −DQB1*0201 positive patients have a significantly higher risk of acute onset of the disease (AOD), radiological stage I erythema nodosum (EN), Löfgren's syndrome, no-medicament therapy, and pulmonary sarcoidosis. On the other hand, the group of non-treated patients (corticosteroids and/or immunosuppressive) showed a significantly lower presence of HLA-B15 antigen in comparison to controls and treated patients ($P = 0.0490$ and $P = 0.0379$, respectively) and for DRB1*04 specificity ($P = 0.0078$ and $P = 0.0065$, respectively). In the group of patients with AOD, those who were positive for DRB1*16 specificity have a statistically significant chance to develop EN, as opposed to those who are positive for DRB1*15 specificity.

## Introduction

Sarcoidosis, found in most populations worldwide, is a multisystem granulomatous disease of unknown etiology that predominantly affects the lungs. Sarcoidosis affects both women and men, but it is slightly more predominant in women. Although, the prognosis of the disease is generally good, and approximately two-thirds of the patients achieve remission, in the remaining patients the disease persists, requires therapy, and may even be fatal for 1%–5% of patients (1). For that reason, it might be very helpful to find markers that are capable of distinguishing different forms of the disease.

The etiology of sarcoidosis is unknown, but the accumulation of CD4+ T lymphocytes and macrophages at the inflammatory sites is suggestive of an immunological reaction (2). Multiple causes of sarcoidosis have been proposed and much evidence support genetic inheritance, infectious transmission, and shared exposure to environmental agents. Current theory suggests that the disease develops in genetically predisposed hosts who are exposed to certain environmental agents that trigger an exaggerated inflammatory immune response leading to granuloma formation (3).

A genetic predisposition to sarcoidosis is proposed by different researches in distinct ethnic groups and by family studies (4). The first suggestion that genetic factors might play a part in the predisposition to sarcoidosis dates to the 1920s when the first cases of acute sarcoidosis in siblings were described. The prevalence of familial clustering in sarcoidosis (range 1.7% – UK to 9.6% – Ireland) has been reported in the past (5). Numerous studies have focused their investigation on the search for genetic factors that

 

influence susceptibility to sarcoidosis in HLA region, although the influence of other genes might also be very important (6). First studies about the association between sarcoidosis and HLA, 20 years ago, reported about HLA-B8 and −DR3 association with sarcoidosis, especially with the acute form of the disease, but also in general population of patients (7). Recently, association of sarcoidosis with variable HLA alleles in different populations has been described, but most of the present studies showed a relationship between sarcoidosis and alleles at HLA-DRB1 and DQB1 loci (8–11). Unfortunately, a consensus about which HLA allele is important in sarcoidosis has not been achieved (12). The heterogeneity of the reported associations between sarcoidosis and HLA could be explained by different clinical categories of the study subjects or by different methods used for the HLA typing, but also with the specificity of each investigated population. First, although sarcoidosis is usually regarded as one disease entity, patients suffering from it constitute a heterogeneous group regarding their clinical features such as symptoms, onset of disease, organ involvement, and prognosis. This represented a problem, especially at the beginning of investigations, when studies were performed on a small number of patients and sometimes on a very heterogeneous group of patients (13, 14). Furthermore, problems resulted from the chosen technique as well because all previous studies used serologic typing techniques instead of molecular typing methods, which give more correct classifications. Finally, population studies among healthy unrelated individuals clearly showed that different populations share the same HLA alleles and haplotypes, but also showed that ethnically specific distribution of HLA alleles and haplotypes exists in various populations.

Additional proof for the role of the HLA region in pathogenesis of sarcoidosis was gained by genome-wide linkage study, which has emphasized the importance of the HLA as candidate genes in disease development, namely the greatest linkage score was found within this part of human genome.

The aim of the present study was to investigate HLA class I and class II polymorphisms in a well-characterized group of the Croatian sarcoidosis patients. In this study, we have also searched for a characteristic combination of HLA alleles and their relationship to the clinical manifestations of sarcoidosis. The current investigation is the first report about the genetic background of sarcoidosis among Croatian patients.

## Materials and methods

### Patients

The sample consisted of 142 patients from University Hospital for Lung Diseases 'Jordanovac', Zagreb, Croatia. There were 80 females and 62 males, with a median age of 42 years (range between 23 and 64). Diagnosis of sarcoidosis was based on consistent clinical features together with biopsy-proven noncaseating epithelioid cell granulomas, according to the international guidelines (15). The clinical symptoms of the patients varied from none (sarcoidosis detected on routine chest radiography) to more or less severe respiratory symptoms or symptoms related to other organ involvement. Data concerning clinical parameters were derived from the patients' medical files. Lung function measurements, including forced expiratory volume in 1 s ($FEV_1$) and forced vital capacity (FVC), were measured with a pneumotachograph. The diffusing capacity for carbon monoxide ($D_{LCO}$) was measured by the single-breath method (Masterlab, Jaeger, Wurzburg, Germany). Values were expressed as a percentage of those predicted. Chest radiographs were graded according to the radiographic staging of DeRemee (0 to III), adding stage IV, the end stage of lung fibrosis (16).

### Controls

A total of 190 unrelated healthy individuals without any signs of the disease served as controls for HLA class I and class II polymorphisms (17). They originate from different regions of Croatia and form a representative sample of the Croatian population.

### Methods

Blood was collected in two test tubes [one with ethylenediaminetetraacetic acid (EDTA) and one with heparin]. Lymphocytes were isolated from heparinized blood by gradient centrifugation and tested for HLA class I (A and B) polymorphisms by microlymphocytotoxicity test. Blood with EDTA was used for isolation of genomic DNA by NucleoSpin kits following the manufacturer's protocol (NucleoSpin, Macherey-Nagel, Düren, Germany). After isolation, DNA samples were tested for HLA class II (DRB1 and DQB1) alleles by PCR-SSP method.

### Statistics

Phenotype frequencies of HLA class I antigens (A and B) and HLA class II alleles (DRB1 and DQB1) were calculated for the patient and control groups. The frequencies of tested HLA loci were determined by direct counting: in cases when only one specificity was present at the given HLA locus, the second specificity was marked as not defined. The chi-squared test was used to compare the expected value of genotype with its observed value in order to confirm whether it satisfies the Hardy–Weinberg law. The significance of the associations between loci was estimated from $2 \times 2$ tables by chi-squared test (Yates' correction) or by Fisher exact test when the number of cases was less than five.


 

The odds ratio (OR) of disease risk for a given marker was also calculated from the $2 \times 2$ contingency. A value of $P < 0.05$ was considered significant.

## Results

One hundred and forty-two patients and matched healthy individuals were recruited for the study. Patient characteristics are listed in Table 1. Eighty-seven (61.3%) had chronic onset of the disease (COD) and 55 (38.7%) patients had acute onset of the disease (AOD). Among patients with AOD, 42 had erythema nodosum (EN). A group of 27 (19.9%) patients had Löfgren's syndrome, while 75 (52.8%) patients developed extrapulmonary manifestations. Approximately half of our group of patients ($n = 78$; 54.9%) required immunosuppressive therapy. Patients were also divided in two groups regarding their lung function: the first group was formed by patients ($n = 85$; 59.9%) for whom the values of $FEV_1$, FVC, and/or $D_{LCO}$ test were less than 80% predicted, while the second group consisted of patients ($n = 57$; 41.1%) for whom the values of any of the three tests were higher than 80% predicted.

Among 16 different observed HLA-A antigens, none was found with significantly increased or decreased frequency in the patient group when compared with the control group. The HLA-B8 was observed with significantly higher frequency among patients in comparison to controls (31.69% *vs* 16.32%; $P = 0.00154$, OR = 2.379). At DRB1 locus, DRB1*0301 allele showed higher phenotype frequency among patients with sarcoidosis than among controls (28.87% *vs* 20.53%; $P$ – marginally significant; OR = 1.626). Thirteen different alleles were observed at DQB1 locus in the patient's group, the most frequent was DQB1*0301 (46.48%), while DQB1*0201 allele was found with the same frequency as DRB1*0301 allele.

Our tested patients with sarcoidosis showed different clinical features such as symptoms, onset of disease, organ involvement, chest X-ray stage, or medicament treatment. Therefore, patients were divided and analyzed regarding some of these parameters. Because in the majority of previous sarcoidosis studies, patients with acute and chronic sarcoidosis were investigated, we also started our analysis with respect to the onset of the disease. Comparison of our patients with acute and chronic onset of sarcoidosis with controls showed some differences (Table 2). The HLA-B8 antigen was significantly increased in the group of patients with acute onset of sarcoidosis only when compared with controls (19.1% *vs* 8.2%; $P = 0.0001$) but not in comparison to the patients with COD (19.1% *vs* 13.2%; $P > 0.05$). The difference in the frequency of HLA-B38 between two groups of patients was marginally significant but not in comparison to the controls. The higher frequency of

**Table 1** Patient characteristics

| | *n* (%) |
|---|---|
| Number of patients | 142 |
| Females | 80 (56.3) |
| Males | 62 (43.7) |
| Biopsy confirmation | 142 (100) |
| Mean age at disease onset | 42 years |
| Onset of disease | |
| Acute | 55 (38.7) |
| Chronic | 87 (61.3) |
| Erythema nodosum | 42 (29.6) |
| Löfgren's syndrome | 27 (19.1) |
| Chest radiograph | |
| Stage I | 57 (40.1) |
| Stage II | 61 (49.2) |
| Stage III | 24 (16.9) |
| Stage IV | 0 |
| Lung function tests | |
| FVC < 80% | 34 (23.9) |
| $FEV_1$ < 80% | 39 (27.5) |
| $D_{LCO}$ < 80% | 71 (50.0) |

$D_{LCO}$, diffusing capacity for carbon monoxide; FVC, forced vital capacity; $FEV_1$, forced expiratory volume in 1 s; HLA, human leukocyte antigen

**Table 2** The distribution of the most frequent HLA-B and −DRB1 specificities among Croatian patients with acute and chronic onset of sarcoidosis and healthy controls

| HLA- | Patients with AOD ($n = 55$) % | Patients with COD ($n = 87$) % | Controls ($n = 190$) % |
|---|---|---|---|
| B7 | 0.0909 | 0.1149 | 0.1684 |
| B8 | 0.4000[a] | 0.1322 | 0.1632 |
| B14 | 0.0182 | 0.0115 | 0.0526 |
| B15 | 0.0182 | 0.1379[b] | 0.1158 |
| B17 | 0.1034 | 0.0364 | 0.0526 |
| B18 | 0.1636 | 0.1724 | 0.2263 |
| B27 | 0.1091 | 0.1379 | 0.1053 |
| B35 | 0.2000 | 0.1724 | 0.2105 |
| B38 | 0.1273 | 0.0460 | 0.1316 |
| B40 | 0.0345 | 0.0727 | 0.0684 |
| B44 | 0.2000 | 0.1839 | 0.1474 |
| B51 | 0.2545 | 0.1839 | 0.2316 |
| DRB1*01 | 0.1636 | 0.1149 | 0.1895 |
| DRB1*0301 | 0.4000[c,d] | 0.2184 | 0.2053 |
| DRB1*04 | 0.1636 | 0.0920 | 0.1579 |
| DRB1*07 | 0.1455 | 0.1034 | 0.2158 |
| DRB1*11 | 0.2545 | 0.4598[e] | 0.3632 |
| DRB1*13 | 0.1818 | 0.1724 | 0.2842 |
| DRB1*15 | 0.2182 | 0.2759[f] | 0.1474 |
| DRB1*16 | 0.2727 | 0.1609 | 0.1737 |

AOD, acute onset of disease; COD, chronic onset of disease; HLA, human leukocyte antigen

[a] $P = 0.0001$ (in comparison to controls)
[b] $P = 0.0330$ (in comparison to patients with COD)
[c] $P = 0.0326$ (in comparison to patients with COD)
[d] $P = 0.0057$ (in comparison to controls)
[e] $P = 0.0228$ (in comparison to patients with COD)
[f] $P = 0.0175$ (in comparison to controls)

 

HLA-B15 antigen among patients with COD when compared with patients with AOD was significant (6.9% *vs* 0.9%; $P = 0.0330$). Among HLA-DRB1 specificity, DRB1*0301 was significantly more present among patients with AOD than among patients with COD (44.0% *vs* 21.8%; $P = 0.0326$) as well as among controls (20.9% *vs* 20.5%; $P = 0.0057$; $P$corr $= 0.0013$). Lower frequency of DRB1*04 specificity among patients with COD did not show significance either in comparison to the control group (9.2% *vs* 15.8%; $P > 0.05$) nor in comparison to the group of patients with AOD (9.2% *vs* 16.4%; $P > 0.05$). The frequency of the DRB1*11 allele at 46.0% among patients with COD was significantly increased when compared with the patients with AOD ($P = 0.0228$), but not in comparison to the control subjects. Patients with COD also showed significantly higher frequency of DRB1*15 specificity in comparison to the controls (27.6% *vs* 14.7%; $P = 0.0175$).

Among 55 patients with AOD, 42 patients presented with EN, while 13 patients did not have EN. Comparison of patients with EN and controls showed significant differences for HLA-B8 antigen ($P = 0.0003$), DRB1*16 ($P = 0.0342$), and DRB1*0301, −DQB1*0201 combination ($P = 0.0044$). It is interesting to note that among 42 patients with EN, 14 patients were positive for DRB1*16 specificity (33.33%), while none of the patients with AOD, but without EN, was positive for this specificity (33.30% *vs* 0%; $P = 0.0121$). At the same time, DRB1*15 allele was significantly increased among 13 patients with AOD and without EN in comparison to patients with EN (53.81% *vs* 11.85%; $P = 0.0037$). Twenty-seven patients with acute onset of sarcoidosis and EN also showed Löfgren's syndrome. In this group of patients, HLA-B8 antigen ($P = 0.0208$), DRB1*16 ($P = 0.0102$), and DRB1*0301, −DQB1*0201 combination ($P = 0.0037$) were also significantly increased when they were compared with the controls.

We also divided patients into two groups according to the medicament treatment: treated and non-treated patients. Non-treated patients did not receive corticosteroids and/or immunosuppressives and they did not have signs of lung or extrathoracic sarcoidosis with organ damage, i.e. severe sarcoidosis. In Table 3, we listed phenotype frequencies of the HLA specificities positively and negatively associated with susceptibility to some other clinical features of sarcoidosis other than the onset of the disease. Patients treated with therapy showed significantly higher phenotype frequency of DRB1*15 specificity ($P = 0.0165$). The non-treated patients showed significantly higher frequency of HLA-B8 antigen ($P = 0.0003$) and DRB1*0301, −DQB1*0201 haplotypic association only in comparison to the controls ($P = 0.00252$). The lower phenotype frequency of HLA-B15 antigen among non-treated patients was significant in comparison to the control group ($P = 0.04897$) and to the treated group of patients ($P = 0.0376$). Lower frequency of HLA-DRB1*01 specificity among treated patients (9.0%) did not reach significant $P$ value ($P$ – marginally significant) in comparison to controls (19.0%). At the same time, lower frequency of DRB1*04 specificity among non-treated patients was statistically significant in comparison to the controls ($P = 0.0078$) as well as in comparison to the treated group of patients ($P = 0.0065$).

The difference in the presence of HLA-B8 antigen and DRB1*0301 allele among patients with chest X-ray stage I and controls was also statistically significant ($P = 0.0039$ and $P = 0.0245$, respectively), while the difference in the distribution of B38 (3.5% *vs* 13.2%) and B15 (3.5% *vs* 11.6%) was marginally significant. At the same time, an increased phenotype frequency with significant $P$ values was found for B8 antigen ($P = 0.0198$) and DRB1*15 ($P = 0.0237$) among patients with chest X-ray stage II or III in comparison to controls. No significant differences in the distribution of HLA alleles among these two subgroups of patients were found.

Finally, we compared patients with only pulmonary sarcoidosis to the patients with systemic disease. A trend of

**Table 3** The list of HLA specificities positively and negatively associated with susceptibility to different clinical features of sarcoidosis

| Patients | HLA- | Patients % | Controls ($n = 190$) % | $P$ | OR |
|---|---|---|---|---|---|
| Treated patients ($n = 78$) | DRB1*15 | 28.21 | 14.74 | <0.05 | 2.27 |
| Non-treated patients ($n = 64$) | B8 | 39.06 | 16.32 | <0.001 | 3.29 |
| | B15 | 3.13 | 11.28 | <0.05 | 0.25 |
| | DRB1*0301, −DQB1*0201 | 40.63 | 20.53 | <0.01 | 2.65 |
| | DRB1*04 | 3.13 | 15.79 | <0.01 | 0.17 |
| Patients with chest X-ray stage I ($n = 57$) | B8 | 35.09 | 16.32 | <0.01 | 2.77 |
| | DRB1*0301, −DQB1*0201 | 35.09 | 20.53 | <0.05 | 2.20 |
| Patients with chest X-ray stage II, III and IV ($n = 85$) | B8 | 33.33 | 16.32 | <0.05 | 2.14 |
| | DRB1*15 | 30.67 | 14.74 | <0.05 | 2.15 |
| Patients with pulmonary sarcoidosis ($n = 75$) | B8 | 33.33 | 16.32 | <0.01 | 2.57 |
| | DRB1*0301, −DQB1*0201 | 37.33 | 20.53 | <0.01 | 2.31 |

HLA, human leukocyte antigen; OR, odds ratio


 

significant difference for HLA-B8 antigen was also observed in patients with pulmonary sarcoidosis compared with the control group ($P = 0.0039$). Statistically significant difference was again reached for DRB1*0301 and DQB1*0201 alleles. These two alleles were also significantly more present among patients with pulmonary sarcoidosis in comparison to the controls ($P = 0.0074$) as well as in comparison with the patients with systemic disease (37.3% *vs* 19.4%; $P = 0.03014$). The higher frequency of DRB1*15 allele in the group of patients with pulmonary sarcoidosis was not significant in comparison to controls.

The study showed that HLA-B8 antigen and DRB1*0301, −DQB1*0201 haplotypic association were significantly increased in some subgroups of sarcoidosis patients. We therefore also investigated which of these genetic markers shows primary and which of them shows secondary association as a result of its linkage disequilibrium (LD) (18). Tests showed that HLA-B8 antigen is not increased when stratified for DRB1*0301 allele, while converse tests showed the same for DRB1*0301 allele. Analyses have proved a well-known significant LD between HLA-B8 antigen and DRB1*0301 allele among both patients and controls (data not presented).

## Discussion

Numerous studies have suggested that there is an association between certain HLA alleles and sarcoidosis. No consensus was established about which HLA locus is directly involved in the pathogenesis of sarcoidosis; various authors suggested that different loci within the HLA region are associated, but the most recent studies mainly focused on the HLA class II genes.

The aim of this first case–control study in the Croatian population was to analyze HLA class I and class II polymorphisms among patients with sarcoidosis and to establish a database for further genetic studies of this very complex disease. Our data clearly showed the existence of an association between HLA-B8 and sarcoidosis in general (regardless of the onset of the disease, EN, Löfgren's syndrome, medicament treatments, or chest X-ray stage) as well as among patients with AOD and radiological stage I. In this study, we also did not observe any statistically significant difference in the distribution (increased or decreased) of HLA-B7 antigen; i.e. authors from Sweden reported that HLA-B*07 and HLA-B*08 independently confer increased risk of sarcoidosis (19).

Our finding that DRB1*0301 is a susceptibility allele for sarcoidosis with AOD is in concordance with data for Scandinavian, German, and Italian populations where a significantly higher frequency of this allele among patients was also observed (19–21). In fact, in those studies DR3, more specifically DR17, was found to be associated with favorable prognosis, spontaneous resolution, and short duration of the disease in different sarcoidosis patient groups.

The difference in the distribution of DRB1*15 allele among our patients with the chronic onset of sarcoidosis and control subjects is similar to observations by authors from Czech Republic, Poland, Sweden, and UK who found an increased occurrence of this allele among patients with chronic course of sarcoidosis (19, 22–24). At the same time, they also reported about the association with DRB1*14 allele, which could not be confirmed with the data from our study. The suggestion of DR1 and DR4 as protective HLA specificities against the development of sarcoidosis was not confirmed by this study either. The only observed statistically significant difference in the distribution of DRB1*04 specificity is that Croatian patients with this allele are more likely not to require corticosteroids and/or immunosuppressive therapy.

One of the highly conserved HLA haplotypes is HLA-B*08, −DRB1*0301, −DQA1*0505, or −DQB1*0201, which also extends to HLA-A*01. Because this is the haplotype with one of the strongest LD observed in the HLA region, it is difficult to establish which allele is primarily associated with the disease. Our analysis showed that both HLA markers, HLA-B8 and DRB1*0301, equally contribute to sarcoidosis, while the association with DQB1 appears secondary as a result of LD. This result supports the thesis that different heterodimers of the associated haplotype probably act together. However, it is also possible that DRB1 locus plays the most important role in sarcoidosis etiology because the majority of T lymphocytes accumulating in the affected organs express the $\alpha/\beta$ T-cell receptor that identifies antigenic peptides in the complex with HLA class II molecules on antigen-presenting cells (25).

Considering the results of the present study, the next logical step in the elucidation of sarcoidosis etiology would be to include the analysis of the region between HLA-B and DRB1 loci in studies about the role of the HLA region in predisposition to sarcoidosis; namely investigations from various populations reported about specific alleles at microsatellites and single-nucleotide polymorphisms (SNP) within the tumor necrosis factor (TNF) gene cluster characteristic for the above-mentioned haplotype. The reason for including this region in investigations of sarcoidosis was provided by a study from the Czech Republic, which reported about the association between two SNPs (TNF-308G/A and lymphotoxin-alpha (LTA)+ 252*A/G) with Löfgren's syndrome (26). Additional support for the investigation of polymorphisms within the TNF gene cluster is a Japanese study, which reported about the association between TNFa2 allele among patients with cardiac sarcoidosis (27, 28).

In summary, these findings have clinical implications in the diagnosis of sarcoidosis, and they might help physicians in classification of disease severity and prognosis

 

prediction at the beginning of the disease. Taken together, our results strongly imply that the HLA-B8, −DRB1*0301, −DQB1*0201 haplotype genetically and primarily determines the predisposition to sarcoidosis in the Croatian population, and patients positive for this haplotype have a higher risk for AOD, radiological stage I, and Löfgren's syndrome, and they probably will not need immunosuppressive therapy.

## References


1. Baughman R, Lower E, du Bois RM. Sarcoidosis. *Lancet* 2003: **361**: 1111–18.
2. Newman LS, Rose CS, Maier LA. Sarcoidosis. *N Engl J Med* 1997: **36**: 1224–34.
3. Normet AM, Saller RD, Parham P, Engelhard VH, Littman DR. Cell-cell adhesion mediated by CD8 and MHC class I molecules. *Nature* 1988: **336**: 79–81.
4. Schurmann M, Lympany PA, Reichel P et al. Familial sarcoidosis is linked to the major histocompatibility complex region. *Am J Respir Crit Care Med* 2000: **162**: 861–4.
5. Rybicki BA, Iannuzzi MC, Frederick MM et al. Familial aggregation of sarcoidosis. A case-control etiologic study of sarcoidosis (ACCESS). *Am J Respir Crit Care Med* 2000: **164**: 861–4.
6. Planck A, Eklund A, Yamaguchi E, Grunewald J. Angiotensin-converting enzyme gene polymorphism in relation to HLA-DR in sarcoidosis. *J Intern Med* 2002: **251**: 217–22.
7. Hedfors E, Lindsrom F. HLA-B8/DR3 in sarcoidosis: correlation to acute onset disease with arthritis. *Tissue Antigens* 1983: **22**: 200–3.
8. Berlin M, Fogdell-Hahn A, Olerup O, Eklund A, Grunewald J. HLA-DR predicts the prognosis in Scandinavian patients with pulmonary sarcoidosis. *Am J Respir Crit Care Med* 1997: **156**: 1601–5.
9. Bogunia-Kubik K, Tomeczko J, Suchnicki K, Lange A. HLA-DRB1*03, DRB1*11 or DRB1*12 and their respective DRB3 specificities in clinical variants of sarcoidosis. *Tissue Antigens* 2001: **57**: 87–90.
10. Sharma SK, Balamurugan A, Pandey RM, Saha PK, Mehra NK. Human leukocyte antigen-DR alleles influence the clinical course of pulmonary sarcoidosis in Asian Indians. *Am J Respir Cell Mol Biol* 2003: **29**: 225–31.
11. Martinetti M, Luisetti M, Cuccia M. HLA and sarcoidosis: new pathogenetic insights. *Sarcoidosis Vasc Diffuse Lung Dis* 2002: **19**: 83–95.
12. Maliarik MJ, Chen KM, Major M et al. Analysis of HLA-DPB1 polymorphisms in African-Americans with sarcoidosis. *Am J Respir Crit Care Med* 1998: **158**: 111–14.
13. Foley PJ, McGrath DS, Puscinska E et al. Human leukocyte antigen DRB1 position 11 residues are a common protective marker for sarcoidosis. *Am J Respir Cell Mol Biol* 2001: **25**: 272–7.
14. Sato H, Grutters JC, Pantelidis P et al. HLA-DQB1*0201 a marker for good prognosis in British and Dutch patients with sarcoidosis. *Am J Respir Cell Mol Biol* 2002: **27**: 406–12.
15. Hunninghake GW, Costabel U, Ando M et al. ATS/ERS/WASOG statement on sarcoidosis. American Thoracic Society/European Respiratory Society/World Association of Sarcoidosis and other Granulomatous Disorders. *Sarcoidosis Vasc Diffuse Lung Dis* 1999: **16**: 149–73.
16. DeRemee RA. The roentgenographic staging of sarcoidosis: historic and contemporary perspectives. *Chest* 1983: **83**: 128–33.
17. Grubić Z, Žunec R, Naipal A, Kaštelan A, Giphart MJ. Molecular analysis of HLA class II polymorphism in Croatians. *Tissue Antigens* 1995: **46**: 293–8.
18. Svejgaard A, Ryder LP. HLA and disease associations: detecting the strongest association. *Tissue Antigens* 1994: **43**: 18–27.
19. Grunewald J, Eklund A, Olerup O. Human leukocytes antigen class I alleles and the disease course in sarcoidosis patients. *Am J Respir Crit Care Med* 2004: **169**: 696–702.
20. Luisetti M, Beretta A, Casali L. Genetic aspects in sarcoidosis. *Eur Respir J* 2000: **16**: 768–80.
21. Grosser M, Luther T, Fuessel M, Bickhardt J, Magdolen V, Baretton G. Clinical course of sarcoidosis in dependence on HLA-DRB1 allele frequencies, inflammatory markers, and the presence of M. tuberculosis DNA fragments. *Sarcoidosis Vasc Diffuse Lung Dis* 2005: **22**: 66–74.
22. Rybicki BA. Sarcoidosis and human leukocyte antigen class I and II genes. *Am J Respir Crit Care Med* 2004: **169**: 665–6.
23. Dubaniewicz A, Szczerkowska Z, Hoppe A. Comparative analysis of HLA class I antigens in pulmonary sarcoidosis and tuberculosis in the same ethnic group. *Mayo Clin Proc* 2003: **78**: 436–42.
24. Rossman MD, Thompson B, Frederick M et al. HLA-DRB1*1101: a significant risk factor for sarcoidosis in blacks and whites. *Am J Hum Genet* 2003: **73**: 720–35.
25. Tazi A, Fajac I, Soler P, Valeyre J, Battesti JP, Hance AJ. Gamma/delta T-lymphocytes are not increased in number in granulomatous lesions of patients with tuberculosis or sarcoidosis. *Am Rev Respir Dis* 1991: **144**: 1373–5.
26. Mrazek F, Holla LI, Hutyrova B et al. Association of tumour necrosis factor-alpha, lymphotoxin-alpha and HLA-DRB1 gene polymorphisms with Lofgren's syndrome in Czech patients with sarcoidosis. *Tissue Antigens* 2005: **65**: 163–71.
27. Takashige N, Naruse TA, Matsumori KM et al. Genetic polymorphisms at the tumour necrosis factor loci (TNFA and TNFB) in cardiac sarcoidosis. *Tissue Antigens* 1999: **54**: 191–9.
28. Grutters JC, Sato H, Pantelidis P et al. Increased frequency of the uncommon tumour necrosis factor -857T allele in British and Dutch patients with sarcoidosis. *Am J Respir Care Med* 2002: **165**: 1119–24.



 