

Human Leukocyte Antigen (HLA) Class I and II Alleles in Turkish Patients with Rheumatic Heart Disease

Fuat Gündođdu¹, Yahya Islamođlu¹, Ibrahim Pirim², Yekta Gurlertop¹, Hasan Dogan², Sakir Arslan¹, Serdar Sevimli¹, Enbiya Aksakal¹, Huseyin Senocak¹

Departments of ¹Cardiology and ²Medical Biology and Genetics, Aziziye Hospital Faculty of Medicine, Ataturk University, Erzurum, Turkey

Background and aim of the study: Rheumatic heart disease (RHD) is often preceded by rheumatic fever (RF). The disease is a multisystem inflammatory condition that develops as a sequel to untreated throat infection by group A beta-hemolytic streptococci. Several studies have suggested that genetic susceptibility to RHD may be linked to human leukocyte antigen (HLA) class II alleles. The study aim was to investigate the association between RHD and the antigens HLA-A, -B, -C, -DR and -DQ profile in RHD patients in eastern Turkey.

Methods: A case-control study was conducted which included 85 unrelated patients with RHD, and 85 control subjects. The diagnosis was supported by echocardiography and histories of RHD of those patients who underwent valve replacement. The association of class I and class II HLA antigens was

Rheumatic fever (RF) occurs as a sequel to throat infection by group A beta-hemolytic streptococci, and is an important health problem in developing countries. Following RF, approximately 30% of patients develop rheumatic heart disease (RHD), which not only has a high morbidity but also burdens the public health system with high costs (1). Taken together, the relatively low attack rate of RF after untreated streptococcal pharyngitis (up to 2-3%), the relatively high concordance rate for RF in monozygotic twins (19%) compared to dizygotic twins (2.5%) (2), and the high familial incidence of RF (3) suggest the involvement of host genetic factors in susceptibility to RF. In RHD, as in most autoimmune diseases, human leukocyte antigen (HLA) class II alleles have shown a stronger corre-

lated in RHD and control subjects using a sequence-specific primer (SSP) method.

Results: The phenotypes HLA-B51, -Cw*4 and -DRB1*01 were encountered in significantly lower frequencies in patients with RHD compared to the control population ($p < 0.05$, $p < 0.05$, $p < 0.05$, respectively). There was also a significant increase in antigen frequency of HLA-DQB1*08 in RHD patients compared to controls ($p < 0.005$).

Conclusion: Among the studied population, the results suggested that susceptibility to RHD was HLA-related, with HLA-DQB1*08 most likely influencing the occurrence of the condition. HLA-B51, -Cw*4 and -DRB1*01 appeared to be more common in control subjects.

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lation risk than class I alleles (4-8). However, an apparent discrepancy has been recognized as to the nature of susceptibility and/or protective alleles, which demonstrate a different distribution among different populations. Variant linkage disequilibrium patterns with HLA-DR or -DQ alleles may also have contributed to these apparent discrepancies. RHD has also been related to HLA-DR3 in Turkish and Indian populations, to -DR7 in Turkish and Brazilian populations (5,6), and to -DR1 in black patients (7), as well as to -DR2 and -DR6 and finally -DQ2 in Indian patients (8). Thus, the study aim was to investigate the role of HLA in susceptibility to RHD in the northeast region of Turkey and to contribute to the data which relate HLA to RHD.

Clinical material and methods

Selection of patients and controls

The study group comprised 85 unrelated patients (55 females, 30 males; mean age 46.9 ± 14.8 years; range: 25 to 70 years). Each patient fulfilled the clinical and echocardiography criteria for RHD or had a history of valve surgery related to rheumatic valve involvement.

Address for correspondence:

Dr. Fuat Gündođdu, Ahmet Sefik Kolaylı Cad., 1. Sok. Yeni Elif Sitesi A2 Blok No: 18, Daire No: 51, Etlik-Keciören, 06020-Ankara, Turkey
Tel: +90 442 3166333
Fax: +90 442 3166340
e-mail: gundogdudr@gmail.com

The updated Jones criteria were used for clinical diagnosis (9). In all participants, M-mode, two-dimensional (2-D) and Doppler analysis (continuous- and pulsed-wave, color flow scanning) was performed according to the recommendations of the American Society of Echocardiography (10). A diagnosis of rheumatic valve involvement was based on following criteria (11,12): elongated chordae to the anterior leaflet, leaflet thickening, annular dilatation, valve prolapse, focal nodularities, and valvular stenosis or any degree of regurgitation.

The characteristics of pathological mitral regurgitation were as follows: assessment of regurgitant jet features; color jet identified in at least two planes; mosaic color jet; and persistence of the jet throughout systole (13). Aortic regurgitation was quantified by the ratio between the length of the regurgitation jet and the left ventricular outflow tract width (14). In valvular stenotic lesions, mitral stenosis was accurately quantified by planimetry of transthoracic 2-D images, the mitral valve area was determined by echo-Doppler measurement of the transvalvular gradients, and valve area estimated using the pressure half-time method.

The control group comprised 85 unrelated normal bone marrow and kidney donors (50 females, 35 males; mean age 43.6 ± 12.2 years; range: 25 to 57 years). The main inclusion criterion for these subjects was an absence of any known HLA-associated disease.

Tissue typing for HLA antigens, both for patients and control subjects, was performed in the same laboratory.

The study was approved by the local ethics committee.

Preparation of DNA from peripheral blood cells

Venous blood samples were removed from each patient or control subject into EDTA tubes, and subsequently used to prepare DNA from the peripheral blood

cells by using the GenElute Kit (Sigma, St. Louis, MO, USA) according to the manufacturer's instructions.

HLA class I and II genotyping

Low-resolution typing for the HLA-A, -B, -C and HLA-DR/DQ was performed using the PCR sequence-specific primer (PCR-SSP) method. For typing, SSP HLA class I generic DNA Typing Tray, Lot 002 and SSP HLA class II generic DNA Typing Tray, Lot 004 (One Lambda, Canoga Park, CA, USA) were used, according to the manufacturer's instructions.

Statistical analysis

Data were presented as mean ± SD. Differences between patients and controls in terms of the frequencies of various HLA antigens were calculated using the standard chi-square method with Yates' correction. P-values were corrected according to Yates' method, and a p-value <0.05 was considered to be statistically significant. The degree of association was calculated using odds ratios (OR).

Results

Clinical features

Among the RHD patients, 52 had echocardiographically proven valvular damage, and 33 underwent valve surgery. The echocardiographic findings of the patient group are listed in Table I.

Gene frequencies

The phenotype frequencies of HLA-A alleles, as defined by the PCR-SSP method, are listed in Table II. The inter-group distribution of HLA-A alleles in the patient and control groups appeared not to be significant.

However, HLA-B51 allele frequency (35.3%) was significantly greater in controls than in the patients

Table I: Echocardiographic findings of valvular lesions (n = 85) in the patients.

Valvular lesion	Mitral valve (n = 60)	Aortic valve (n = 7)	Aortic+mitral valves (n = 15)	Triple-valve (n = 3)
Commissural fusion, focal nodularities, or leaflet thickening	3 (4)	1 (1)	2 (2)	0 (0)
Stenosis	25 (30)	1 (1)	0 (0)	0 (0)
Regurgitation	7 (8)	1 (1)	0 (0)	0 (0)
Stenosis+Regurgitation	5 (6)	1 (1)	1(1) (MS+AR) 2 (2) (MR+AR)	2 (2) (MR+AR+TS) 1 (1) (MS+AR+TR)
Valve replacement	20 (23)	3 (4)	10 (12)	0 (0)

Values in parentheses are percentages.

AR: Aortic regurgitation; AS: Aortic stenosis; MR: Mitral regurgitation; MS: Mitral stenosis; TR: Tricuspid regurgitation; TS: Tricuspid stenosis.

Table II: Phenotype frequencies of HLA-A alleles in Turkish patients with rheumatic heart disease and in controls.

HLA subtype	Controls (n = 85)	Patients (n = 85)
A*1	13 (15.3)	19 (22.4)
A*2	35 (41.2)	37 (43.5)
A*3	23 (27.1)	16 (18.8)
A*11	9 (10.6)	10 (11.8)
A*23	4 (4.7)	5 (5.9)
A*24	24 (28.2)	29 (34.1)
A*25	0 (0)	1 (1.2)
A*26	7 (8.2)	7 (8.2)
A*29	4 (4.7)	9 (10.6)
A*30	6 (7.1)	5 (5.9)
A*31	3 (3.5)	2 (2.3)
A*32	8 (9.4)	5 (5.9)
A*33	5 (5.9)	6 (7.1)
A*36	0 (0)	2 (2.4)
A*66	6 (7.1)	2 (2.4)
A*68	9 (10.6)	8 (9.4)
A*74	1 (1.2)	0 (0)

Values in parentheses are percentages.

Table III: Phenotype frequencies of HLA-B alleles in Turkish patients with rheumatic heart disease, and in controls.

HLA subtype	Controls (n = 85)	Patients (n = 85)
B*7	5 (5.9)	8 (9.4)
B*8	5 (5.9)	12 (14.1)
B*13	4 (4.7)	1 (1.2)
B*14	3 (3.5)	7 (8.2)
B*15	0 (0)	0 (0)
B*18	4 (4.7)	5 (5.9)
B*27	11 (12.9)	7 (8.2)
B*35	26 (30.6)	24 (28.2)
B*37	1 (1.2)	1 (1.2)
B*38	6 (7.1)	9 (10.6)
B*39	3 (3.5)	4 (4.7)
B*41	5 (5.9)	4 (4.7)
B*44	13 (15.3)	10 (11.8)
B*49	8 (9.4)	7 (8.2)
B*50	7 (8.2)	10 (11.8)
B*51	30 (35.3)	14 (16.5) [§]
B*52	8 (9.4)	10 (11.8)
B*54	1 (1.2)	3 (3.5)
B*55	5 (5.9)	4 (4.7)
B*57	2 (2.3)	8 (9.4)
B*58	2 (2.3)	1 (1.2)
B*61	1 (1.2)	1 (1.2)
B*62	3 (3.5)	1 (1.2)
B*63	4 (4.7)	1 (1.2)

Values in parentheses are percentages.

[§] $\chi^2_{\text{Y}} = 6.89$; p-value = 0.009; Odds ratio = 0.362; CI (95%) = 0.175-0.747.

(16.5%, p = 0.009, OR = 0.362, 95% CI = 0.175-0.747) (Table III).

The frequencies of HLA-C alleles are listed in Table IV. HLA-Cw*4 was encountered at a significantly lower frequency (16.5%) in RHD patients compared to controls (32.9%, p = 0.021, OR = 0.401, 95% CI = 0.193-0.883).

Details of genotyping of HLA class II alleles are listed in Tables V and VI. HLA-DRB1*01 frequency was significantly lower in RHD patients than in controls (3.5% versus 16.5%, p = 0.011, OR = 0.186, 95% CI = 0.051-0.672). The frequency of HLA-DQB1*08 allele was

Table IV: Phenotype frequencies of HLA-C alleles in Turkish patients with rheumatic heart disease, and in controls.

HLA subtype	Controls (n = 85)	Patients (n = 85)
Cw*1	7 (8.2)	5 (5.9)
Cw*2	14 (16.5)	6 (7.1)
Cw*3	6 (7.1)	7 (8.2)
Cw*4	28 (32.9)	14 (16.5) [§]
Cw*5	5 (5.9)	5 (5.9)
Cw*6	19 (22.4)	14 (16.5)
Cw*7	23 (27.1)	0 (0)
Cw*17	2 (2.4)	0 (0)

Values in parentheses are percentages.

[§] $\chi^2_{\text{Y}} = 5.34$; p-value = 0.021; Odds ratio = 0.401; CI (95%) = 0.193-0.883.

Table V: Phenotype frequencies of HLA-DR alleles in Turkish patients with rheumatic heart disease, and in controls.

HLA subtype	Controls (n = 85)	Patients (n = 85)
HLA-DRB1*01	14 (16.5)	3 (3.5) [§]
HLA-DRB1*03	0 (0)	0 (0)
HLA-DRB1*04	22 (25.9)	29 (34.1)
HLA-DRB1*07	10 (11.8)	15 (17.6)
HLA-DRB1*08	2 (2.3)	3 (3.5)
HLA-DRB1*09	2 (2.3)	0 (0)
HLA-DRB1*10	2 (2.3)	4 (4.7)
HLA-DRB1*11	28 (32.9)	33 (38.8)
HLA-DRB1*13	21 (24.7)	22 (25.9)
HLA-DRB1*14	3 (3.5)	7 (8.2)
HLA-DRB1*15	14 (16.5)	21 (24.7)
HLA-DRB1*16	7 (8.2)	6 (7.1)
HLA-DRB1*17	9 (10.6)	15 (17.6)

Values in parentheses are percentages.

[§] $\chi^2_{\text{Y}} = 6.536$; p-value = 0.011; Odds ratio = 0.186; CI (95%) = 0.051-0.672.

Table VI: Phenotype frequencies of HLA-DQ alleles in Turkish patients with rheumatic heart disease, and in controls.

HLA subtype	Controls (n = 85)	Patients (n = 85)
HLA-DQB1*02	18 (21.2)	28 (32.9)
HLA-DQB1*03	8 (9.4)	2 (2.3)
HLA-DQB1*04	1 (1.2)	2 (2.3)
HLA-DQB1*05	20 (23.5)	21 (24.7)
HLA-DQB1*06	29 (34.1)	29 (34.1)
HLA-DQB1*07	38 (44.7)	38 (44.7)
HLA-DQB1*08	10 (11.8)	26 (30.6) [§]
HLA-DQB1*09	2 (2.3)	0 (0)

Values in parentheses are percentages.

[§] $\chi^2_{29} = 7.929$; p-value = 0.005; Odds ratio = 3.3; CI (95%) = 1.5-7.4.

significantly greater in patients than in controls (30.6% versus 11.8%, p = 0.005, OR = 3.3, 95% CI = 1.5-7.4).

Discussion

Although the results of several studies have suggested that HLA class II antigens are linked to a genetic susceptibility for RHD (6,15-18), these data have demonstrated some inconsistency, with discrepancies among the different studied populations. For example,

Falk et al. (19) reported that the frequency of HLA-A3 was lower among their patient group, whereas Caughey et al. (20) reported the frequency to be higher. In the present study, the frequency of HLA-A alleles was similar in both patients and controls.

It has been reported by Ozkan et al. (5) that the frequency of HLA-B16 was higher among RHD patients, whereas Naito et al. (21) reported a lower frequency among their patients. Among the present patients and controls, there was no significant association between RHD and the frequency of HLA-B16, although HLA-DRB51 was associated with a significantly lower frequency in RHD patients than in controls.

In a separate study, Olmez et al. (22) examined the distribution of class I and class II HLA antigens in 100 Turkish patients with RF, 77 of whom had cardiac involvement. The frequency of the HLA-Cw*2 antigen was significantly higher in patients without cardiac involvement than in those with RF (p <0.05). Among the present patients, no significant association was found between RHD and the frequency of HLA-Cw*2, but the HLA-Cw*4 antigen frequency was significantly higher in controls than in patients.

Rajapakse et al. (18) described a higher HLA-DR4 frequency among Saudi Arabian patients with RHD, while Anastasiou-Nana et al. (16) defined HLA-DR4 as a genetic marker of RHD in US Caucasians. Although the HLA-DR4 allele frequency was high among the present patients, there appeared to be no significant

Table VII: Previously reported HLA class II associations in patients with rheumatic fever and rheumatic heart disease.

Population	Reference	HLA Class II association		Method	Patients (n)	Controls (n)
		Risk	Protection			
Caucasian (America)	Anastasiou-Nana et al. (16)	DR4	DR6	Serology	33	82
Black (America)	Ayoub et al. (17)	DR2		Serology	30	64
Caucasian (America)	Ayoub et al. (17)	DR4		Serology	15	285
Black (South Africa)	Maharaj et al. (7)	DR1, DR6		Serology	120	220
Saudi	Rajapakse et al. (18)	DR4		Serology	25	100
North India	Jhinghan et al. (23)	DR3		Serology	134	400
North India	Taneja et al. (8)	DR3, DQw2	DR2	Serology	54	163
Brazilian	Guilherme et al. (6)	DR7, DR53		Serology	40	118
Brazilian	Weidebach et al. (15)	DR53, DR16(2)		RFLP	24	47
Turkish	Ozkan et al. (5)	DR3, DR7	DR5	Serology	107	203
Mexican	Debaz et al. (4)	DRB1*0301 DRB1*0701 DQB1*0201		PCR-SSO	62	175
Mexican	Hernandez-Pacheco et al. (31)	DRB1*1602 DR16-DQA1		PCR-SSP PCR-SSO	99	98
Japanese	Koyanagi et al. (36)	DRB1*1405 DQA1*0104 DQB1*0503		PCR-SSO	72	525

PCR-SSO: PCR sequence-specific oligonucleotide; PCR-SSP: PCR sequence-specific primer; RFLP: Restriction fragment-length polymorphism.

association with RHD.

Both, Taneja et al. (8) and Jhinghan et al. (23) have described a lower HLA-DR2 frequency in Indian patients with RHD, although this conflicts with the findings of Ayoub et al. (17), who noted that susceptibility to RF in a studied population was HLA-related, with HLA-DR2 conferring protection. Among the present patients, HLA-DRB1*01 was encountered in a significantly lower frequency in RHD patients than in control subjects.

It has been suggested by Hallioglu et al. (24) that the HLA-DQA1*03 allele might serve as a protective factor in Turkish children with RF, although the control group in this study comprised only children and RF may have been detected in controls after childhood.

Gu et al. (25) suggested that DQA1*0101 might contribute towards a genetic susceptibility for RF or RHD in Guangdong Hans, while the DQA1*0102 allele might serve as a resistance marker. The genotyping of HLA-DQA1 may provide a scientific basis for identifying those individuals who are susceptible to RF or RHD. The use of PCR-polyacrylamide gel electrophoresis and silver dyeing to provide a new genotyping method for HLA-DQA1, which is simple to use, sensitive and precise, was established and applied.

In another study, Maharaj et al. (26) was unable to identify any significant differences between patients and controls in terms of HLA-A, -B, -DR and -DQ frequencies. Neither was the role of genetically determined immune response factors in the pathogenesis of chronic rheumatic heart disease evident in this study.

Previous data on RHD suggest that the strong association with HLA alleles would be a function of the genetic heterogeneity of the study population. The strongest associations have been detected among East Indian samples, and the weakest among American whites, who are clearly a genetically heterogeneous population (27). The Mexican Mestizo population is a well-characterized ethnic group, from a genetic point of view, with proportions of 56% Amerindian genes, 40% Caucasian genes, and 4% black genes (28). In the population reported by Hernandez-Pacheco et al. (31), the allele associated with such susceptibility was DR16 (DRB1*1602) - that is, an allele with a high frequency in the majority of North American Amerindians studied, which presents a strong linkage disequilibrium with the DQB1*0301 allele (29,30). These data suggest that the genetic susceptibility to RHD in the Mexican population was not acquired by admixture with Caucasian populations, and it indeed may be possible that the early Americans possessed the susceptibility gene (31).

In the present study, HLA allele distribution for class I was similar in both control subjects and RHD patients. As with class II HLA, some HLA-DR alleles were identified much more often among control sub-

jects, and the HLA-DQB1*08 allele appeared frequently in RHD. This elevated frequency of HLA-DQB1*08 was due to a genetic linkage of HLA-DR alleles such as HLA-DRB1*04.

Although the mechanism by which HLA genes determine susceptibility to RHD is not completely understood, it is well known that major histocompatibility complex genes regulate immune response to infections, bind and present antigens to T cells, and have an important role in T-cell repertoire selection. Thus, molecular mimicry has been proposed as a potential mechanism for streptococcal sequelae leading to RHD. Consequently, individuals with susceptible HLA alleles may present cross-reactive peptides and develop the disease, whereas those with protector HLA alleles would not. This concept argues that a microbial peptide with a certain degree of homology to a self-peptide can stimulate pathogenic self-reactive specific T cells to cause an autoimmune disease in genetically susceptible individuals (31). This study provided an analysis of HLA-A, -B, -C, -DR and -DQ polymorphisms in control and RHD groups, and the allelic distributions or allele carrier frequencies were compared between RHD patients and healthy controls.

In the present study, HLA class I and II allele frequencies were determined in patients of Turkish origin. These alleles show high variability of distribution among different ethnic populations (Table VII), although these differences may also be explained by methodological variation among studies. The most frequently used method, serological HLA genotyping, has several limitations in terms of accuracy and allele subgroup discrimination (32). In the present study, a PCR method was selected on the basis of its accuracy and discriminatory power (33,34). It is possible that the present results might also have been affected by patient selection criteria. For example, Donadi et al. (35) selected their study population from the patients with Sydenham's chorea, whereas the present patients had echocardiographically proven valvular disease.

In conclusion, the results of the present study indicate that susceptibility to RHD identified in the Turkish population is essentially HLA class II- and poorly class I-mediated, with HLA-B51, Cw*4 and DRB1*01 alleles having a greater distribution in control subjects, and DQB1*08 influencing susceptibility. Although the present results were at variance with those of other studies, they have provided information regarding the association of HLA class I and class II alleles with RHD. Unfortunately, inconsistencies in the assessment of HLA class I and class II allele frequencies in RHD patients of different ethnicities render somewhat questionable the possible development of a peptide-based vaccine to combat this disorder.

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