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Comprehensive analysis of the HLA class I and the HLA class II Alleles in Patients with Takayasu Arteritis: Relationship with Clinical Patterns of the Disease and Prognosis

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ABSTRACT

Background: Takayasu arteritis (TA) is a systemic vasculitis, affecting mainly the aorta and its branches.

Objective: To analyze the HLA class I and class II alleles in patients with TA and explore their relationship with clinical and demographic characteristics, and potential significance in prognosis.

Methods: Twenty-five, unrelated TA patients were genotyped for HLA-A, HLA-B, HLA-C, HLA-DRB1, and the HLA-DQB1 loci. The frequencies of the HLA-A, HLA-B, and the HLA-DRB1 were compared with a control group of 1992, while the HLA-C and the HLA-DQB1 were compared with a group of 159 healthy, unrelated individuals.

Results: Among TA patients, 5/25 (20%) were identified as the HLA-B*52 carriers. There was a significant difference in the HLA-B*52 allele frequency in the TA patients (10%) compared with the healthy controls (1.2%). Moreover, presence of the HLA-B*52 was associated with significantly earlier disease onset, more severe clinical presentations, and a poorer response to treatment. The HLA-C*03 was detected in 32% of patients and was present exclusively in those with a clinically mild form of the TA, indicating a putative protective effect.

Conclusion: These findings indicate that the HLA-B*52 allele contributes to a higher susceptibility to the TA whereas the HLA-C*03, can be a protective factor in the TA.

Keywords: Takayasu arteritis, Vasculitis, HLA, Immunogenetics, Biomarkers

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INTRODUCTION

Takayasu arteritis (TA) is a rare systemic vasculitis, affecting the aorta and its branches, with possible involvement of the pulmonary arteries (1). The prevalence rate of the TA varies worldwide: from 40 cases per million reported from Japan to 0.9 per million inhabitants in the USA. The prevalence rate in Europe was estimated at 4.7 to 33 cases per million, and it is explained by the influence of geographical and genetic differences among European populations (2). Although the etiopathogenesis has not been clearly understood yet, accumulating evidence supports the role of genetic factors in the TA development (3). One of the most studied is the association of the presence of human leukocyte antigen class B (HLA-B) alleles and the TA susceptibility (4). The HLA of both classes: class I (HLA-A, HLA-B, and HLA-C) and class II (HLA-DR, HLA-DQ, and HLA-DP) are molecules specialized for the presentation of peptide antigens. It is well-known that particular HLA alleles are associated with certain diseases, but the underlying mechanisms have not been fully explained (5). There have been studies reporting the association between the HLA-B*52 and the TA across different ethnicities: Japanese, Chinese, Thai, Korean, Mexican, Indian, Greek, Turkish, Italian, and North Americans (6–14). Recently, the HLA-B*67:01 was identified in the TA patients in Japan, independently from the presence of the HLA-B*52:01 (15). There are very few studies that have analyzed the association between the HLA Class II alleles and the TA. Although higher frequency of the HLA-DRB1*07 was found among the TA patients in the Chinese Han population, the subsequent analysis failed to prove its association with clinical manifestations of the disease (16). A strong association between the HLA-DPB1*09 and the HLA-DRB1*15 and the TA in Japanese was explained through the HLA-B*52 susceptibility, and its high linkage disequilibrium (LD) with both alleles (17).

The interest in the linkage between the presence of specific HLA alleles and the TA has started growing again, when, in a recently published study, authors proposed a hypothesis that microbiome may also contribute to the disease initiation in genetically susceptible HLA-B*52 positive carriers (18).

We aimed to analyze the HLA class I and class II alleles in patients with the TA and to explore their relationship with clinical and demographic characteristics and potential significance in prognosis. Very few studies have analyzed the genetic background of the TA patients in Europe. To our knowledge, the present study is the first of such a kind in Serbia and West Balkan countries, while the analysis of the HLA profile within European TA patients has been only published by Karageorgaki et al., so far (13).

MATERIALS AND METHODS

Subjects

Twenty- five unrelated patients diagnosed with the TA between January 2007 and May 2019 at the Clinic of Allergy and Immunology - University Clinical Center of Serbia, were studied. The TA diagnosis was based on clinical assessment, laboratory tests, and imaging studies, following the classification criteria for the TA, proposed by the American College of Rheumatology (ACR) in 1990, for adults, and the EULAR/PRINTO/PRES classification criteria for childhood TA: Ankara 2008, for two pediatric patients (19, 20).

The samples of blood were collected between May 2017 and May 2019 from all of the TA patients who signed the informed consent for participating in the study, and the HLA typing was performed.

The study protocol was approved by the Ethics Committee of the University Clinical Center of Serbia, and the Ethics Committee of the Faculty of Medicine - University of Belgrade.

Demographic and Clinical Characteristics

Demographic data, clinical and laboratory characteristics of the disease at the moment of the diagnosis, vascular involvement, type of vascular lesions, heart involvement, presence of the aortic valve dysfunction, hypertension, development of ischemic complications (stroke, myocardial infarction, severe limb ischemia), medications, type of performed surgery or endovascular procedure and values of the inflammatory parameters (sedimentation rate, C-reactive protein, fibrinogen level) were collected from the patients' records. Angiographic type of the disease, based on classification defined by Hata et al. (21), was determined according to the MRI or CT angiography. Additional data about the vascular involvement were obtained by the heart ultrasound, carotid, and vertebral artery Doppler ultrasound.

HLA Typing

The total DNA from the peripheral blood samples was extracted, using the automated system with Maxwell 16 Purification Kit (Maxwell, Promega, Madison, USA). Allelic groups of the HLA-A*, the HLA-B*, the HLA-C*, theHLA-DRB1*, and the HLA-DQB1* loci were typed by the polymerase chain reaction (PCR) sequence-specific oligonucleotide (SSO) probe and PCR products were detected using a Luminex™ platform (One Lambda Inc., Canoga Park, USA). In brief, the target DNA was PCR amplified with the HLA group-specific primers, biotinylated, and detected by R-Phycoerythrin (PE)-conjugated Streptavidin. After denaturation, amplified/biotinylated DNA was hybridized to complementary DNA probes conjugated to fluorescently code microspheres. Each microsphere PE Fluorescent intensity was identified by a Luminex flow analyzer. The determination of the HLA alleles was based on the reaction pattern of the various beads in comparison to the patterns with the known HLA alleles using the HLA Fusion™ Softver version 4.1.

The frequencies of the HLA-A*, the HLA-B*, and the HLA-DRB1* allelic groups were compared with a Serbian population consisting of randomly selected, unrelated, 1992 healthy control subjects who were recruited for the Serbian Bone Marrow (BM) Donor Registry, between 2006 and 2010. The HLA-C* and the HLA-DQB1* allele frequencies were compared with the frequencies obtained from a subgroup of 159 BM donors who were typed for the HLA-C*and the HLA-DQB1* (22).

Statistical Analysis

The frequencies of the HLA alleles and the haplotypes were calculated by direct counting. The significance of the deviations from the Hardy-Weinberg (H-W) equilibrium on each locus was estimated with the R package "genetics" (23). The differences between the observed and expected allele and haplotype frequencies were analyzed by performing 2X2 contingency tables and using the Fisher exact test. To assess the strength of association between the HLA alleles, haplotypes, and the TA, odds ratio (OR) with 95% confidence intervals were calculated; for all statistical tests, the P value<0.05 was considered significant. The Benjamini-Hochberg method was used for P value correction for multiple comparisons. The association between the HLA-B*52 and the clinical covariates was tested using the ordinal logistic regression, Chi-Square, and Fisher's exact test. Genetic Power Calculator, provided as a web application, was used for the *Post hoc* study power analysis (24), assuming α=0.05, and a case to control the ratio of 25:1992, with a difference of the HLA-B*52 allele frequencies as the main finding. The power of the study was estimated at 0.5.

RESULTS

From January 2007 to May 2019, 27 patients were diagnosed with the TA at the Clinic of Allergy and Immunology –University Clinical Center of Serbia. One of the patients died

and one patient did not accept to participate in the study. So, 25 TA patients, 23 females and two males, (92% and 8%, respectively) were included in the study. Demographics, clinical manifestations, the clinical course of the disease, and treatment are shown in Table 1.

We analyzed the presence of the specific HLA class I (HLA-A*, HLA-B*, and HLA-C*) and the HLA class II (HLA-DRB1*, and HLA-DQB1*) groups of alleles in the patients' cohort, and compared the observed frequencies to the expected frequencies obtained from the previously defined control groups (22) (Tables 2 and 3).

We found a significant association between the HLA-B*52 and our TA patients: 20% of our patients (5/25) compared to 2.2% (44/1992) in the control group, were the HLA-B*52 carriers. The frequency of the HLA-B*52 allele in the TA patients was 10% (5/50) and it was significantly higher than in the healthy controls [1.2%; 46/3984, homozygotes frequency=2; P=0.0004, P^{adj}=0.011; OR=9.512 95% CI=3.610-25.071].

However, the statistically significant association initially detected between some HLA class I alleles that might confer susceptibility: the HLA-A*32 (P=0.012; $P^{adjusted}$ (P^{adj})=0.200), the HLA-B*15 (P=0.012; P^{adj}=0.326) and the HLA-B*57 (P=0.018; P^{adj}=0.483), and putative protective factor for the TA: HLA-C*03 (P=0.009; P^{adj}=0.121), did not reach statistical significance after the P value corrections (Table 2).

Since the HLA-B*52 was the only allele found to be statistically higher in our TA patients when compared to controls, we further explored how clinical characteristics of TA differed according to its presence (Table 1).

Patients with HLA-B*52 had a statistically

Table 1. Clinical characteristics of patients

	Total	HLA B52		P value
		Yes (n=5)	No (n=20)	
Age (yrs)	45.5±16.3	24.6±10.8	50.7±13.0	<0.001 [b]
Age of Dg > 40 yrs	16 (64%)	2 (40%)	14 (70%)	0.312 [a]
Angiograph. Type				
I	1 (4%)	0	1 (5%)	
II	13 (52%)	0	13 (65%)	
III	1 (4%)	1 (20%)	0	0.019 [a]
IV	1 (4%)	0	1 (5%)	
V	9 (36%)	4 (80%)	5 (25%)	
Angiographic Type V	9 (36%)	4 (80%)	5 (25%)	0.040 [a]
Aortic regurgitation	18 (72%)	4 (80%)	14 (70%)	1.000 [a]
Remission	19 (76%)	2 (40%)	17 (85%)	0.070 [a]
Remission				
No	6 (24%)	3 (60%)	3 (15%)	0.042 [c]
Yes, with Th	15 (60%)	2 (40%)	13 (65%)	
Yes, without Th	4 (16%)	0	4 (20%)	
Inflammation at diagnosis	23 (92%)	5 (100%)	18 (90%)	1.000 [a]
Glucocorticoids	22 (88%)	5 (100%)	17 (85%)	1.000 [a]
Immunosuppressives	11 (44%)	5 (100%)	6 (30%)	0.009 [a]
Vascular Intervention	6 (24%)	0	6 (30%)	0.289 [a]
Stenosis	20 (80%)	5 (100%)	15 (75%)	0.544 [a]
Aneurysm	4 (16%)	1 (20%)	3 (15%)	1.000 [a]
Vascular complications	7 (28%)	2 (40%)	5 (25%)	0.597 [a]
Hypertension	18 (72%)	3 (60%)	15 (75%)	0.597 [a]

Results are presented as count (%) or mean±standard deviation; [a]Fisher's Exact test [b]t test [c]Mantel-Haenszel chi square test for trend

Table 2. HLA-A*, -B*and -C* allele frequencies in TA patients and controls

Allelic group	TA (n=50) Allele Frequency N (%)	Controls (n=3984) Allele Frequency N (%)	P value[a]	P^{adj}	OR	CI (95%)
A*01	3 (6.00)	568 (14.26)	0.082	0.986	0.384	0.119-1.237
A*02	12 (24.00)	1174 (29.47)	0.408	0.986	0.756	0.394-1.451
A*03	4 (8.00)	451 (11.32)	0.488	0.986	0.685	0.245-1.912
A*11	5 (10.00)	244 (6.12)	0.278	0.986	1.703	0.670-4.329
A*23	1 (2.00)	89 (2.23)	0.986	0.986	0.893	0.122-6.540
A*24	8 (16.00)	442 (11.09)	0.286	0.986	1.526	0.712-3.272
A*25	2 (4.00)	105 (2.63)	0.530	0.986	1.539	0.369-6.418
A*26	4 (8.00)	240 (6.02)	0.540	0.986	1.356	0.484-3.800
A*29	1 (2.00)	29 (0.73)	0.366	0.986	2.783	0.372-20.841
A*30	1 (2.00)	84 (2.11)	0.942	0.986	0.947	0.129-6.943
A*31	0	77 (1.93)	0.380	0.986	0.499	0.030-8.165
A*32	7 (14.00)	190 (4.77)	0.012	0.200	3.250	1.443-7.322
A*33	0	101 (2.53)	0.280	0.986	0.379	0.023-6.183
A*66	0	15 (0.37)	0.830	0.986	2.535	0.149-42.960
A*68	2 (4.00)	168 (4.22)	0.983	0.986	0.946	0.228-3.927
A*69	0	7 (0.18)	0.916	0.986	5.250	0.296-93.183
B*07	1 (2.00)	195 (4.97)	0.374	0.988	0.396	0.054-2.887
B*08	2 (4.00)	344 (8.63)	0.247	0.988	0.440	0.106-1.822
B*13	1 (2.00)	131 (3.29)	0.697	0.988	0.600	0.082-4.380
B*14	0	129 (3.16)	0.203	0.988	0.295	0.018-4.804
B*15	6 (12.00)	144 (3.66)	0.012	0.326	3.636	1.525-8.671
B*18	4 (8.00)	386 (9.89)	0.735	0.988	0.810	0.290-2.264
B*27	1 (2.00)	199 (4.99)	0.359	0.988	0.388	0.053-2.825
B*35	5 (10.00)	516 (13.12)	0.566	0.988	0.747	0.295-1.890
B*37	0	53 (1.33)	0.514	0.988	0.727	0.044-11.948
B*38	1 (2.00)	221 (5.62)	0.287	0.988	0.347	0.048-2.528
B*39	3 (6.00)	124 (3.14)	0.278	0.988	1.987	0.610-6.471
B*40	3 (6.00)	143 (3.61)	0.376	0.988	1.714	0.527-5.574
B*41	0	53 (1.33)	0.514	0.988	0.728	0.044-11.948
B*44	3 (6.00)	360 (9.16)	0.488	0.988	0.642	0.199-2.075
B*45	0	4 (0.10)	0.951	0.988	8.758	0.465-164.829
B*46	0	1 (0.02)	0.988	0.988	26.293	1.058-653.282
B*47	0	19 (0.5)	0.789	0.988	2.013	0.119-33.808
B*48	0	4 (0.10)	0.951	0.988	8.758	0.465-164.829
B*49	1 (2.00)	117 (2.94)	0.792	0.988	0.675	0.092-4.927
B*50	2 (4.00)	45 (1.13)	0.134	0.988	3.647	0.800-15.466
B*51	5 (10.00)	503 (12.85)	0.612	0.988	0.769	0.304-1.946
B*52	5 (10.00)	46 (1.18)	0.0004	0.011	9.512	3.610-25.071
B*53	0	9 (0.23)	0.894	0.988	4.143	0.237-72.159
B*54	0	1 (0.02)	0.988	0.988	26.293	1.058-653.282
B*55	1 (2.00)	45 (1.13)	0.549	0.988	1.786	0.241-13.220
B*56	0	45 (1.13)	0.568	0.988	0.857	0.052-14.109
B*57	4 (8.00)	75 (1.88)	0.018	0.483	4.532	1.590-12.912
B*58	2 (4.00)	34 (0.85)	0.082	0.988	4.840	1.130-20.723

Allelic group	TA (n=50) Allele Frequency N (%)	Controls (n=318) Allele Frequency N (%)	P value	P^{adj}	OR	CI (95%)
C*01	1 (2.00)	22 (6.92)	0.183	0.751	0.275	0.036-2.083
C*02	4 (8.00)	22 (6.92)	0.751	0.751	1.170	0.386-3.549
C*03	9 (18.00)	19 (5.97)	0.009	0.121	3.454	1.465-8.144
C*04	5 (10.00)	43 (13.52)	0.517	0.751	0.711	0.267-.890
C*05	3 (6.00)	11 (3.46)	0.400	0.751	1.781	0.479-6.622
C*06	6 (12.00)	32 (10.06)	0.658	0.751	1.218	0.482-3.082
C*07	5 (10.00)	79 (24.84)	0.015	0.198	0.336	0.129-0.876
C*08	0	13 (4.09)	0.145	0.751	0.224	0.013-3.828
C*12	10 (20.00)	43 (13.52)	0.240	0.751	1.598	0.745-3.432
C*14	1 (2.00)	12 (3.77)	0.597	0.751	0.520	0.066-4.092
C*15	1 (2.00)	15 (4.71)	0.670	0.751	0.412	0.053-3.191
C*16	2 (4.00)	1 (0.31)	0.052	0.624	13.208	1.175-148.482
C*17	0	5 (1.57)	0.479	0.751	0.564	0.030-10.363
C*18	1 (2.00)	1 (0.31)	0.272	0.751	6.469	0.398-105.132

[a]Fisher's Exact test; Significant difference between TA patients and controls is bolded; P^{adj}: P adjusted; OR: Odds Ratio; CI: Confidence interval.

Table 3. HLA-DRB1* and DQB1* allele frequencies in TA patients and controls

Allelic group	TA (n=50) Allele Frequency N (%)	Controls (n=3984) Allele Frequency N (%)	P value[a]	P^{adj}	OR	CI (95%)
DRB1*01	3 (6.00)	410 (10.29)	0.332	0.891	0.556	0.172-1.796
DRB1*03	5 (10.00)	433 (10.87)	0.891	0.891	0.911	0.360-2.308
DRB1*04	6 (12.00)	358 (8.99)	0.454	0.891	1.381	0.585-3.263
DRB1*07	7 (14.00)	284 (7.13)	0.090	0.891	2.121	0.945-4.757
DRB1*08	1 (2.00)	123 (3.09)	0.749	0.891	0.640	0.088-4.677
DRB1*09	0	15 (0.38)	0.829	0.891	2.535	0.150.42.961
DRB1*10	8 (4.00)	44 (1.10)	0.129	0.891	3.731	0.879-15.823
DRB1*11	7 (14.00)	673 (16.89)	0.614	0.891	0.800	0.359-1.788
DRB1*12	0	76 (1.91)	0.384	0.891	0.505	0.030-8.275
DRB1*13	4 (8.00)	527 (13.23)	0.283	0.891	0.570	0.204-1.591
DRB1*14	3 (6.00)	216 (5.42)	0.803	0.891	1.113	0.344-3.606
DRB1*15	7 (14.00)	392 (9.84)	0.336	0.891	1.491	0.666-3.338
DRB1*16	5 (10.00)	433 (10.87)	0.891	0.891	0.911	0.360-2.308
Allelic group	**TA (n=50) Allele Frequency N (%)**	**Controls (n=318) Allele Frequency N (%)**	**P value**	**P^{adj}**	**OR**	**CI (95%)**
DQB1*02	9 (18.00)	54 (16.98)	0.839	0.917	1.073	0.493-2.338
DQB1*03	17 (34.00)	106 (33.33)	0.917	0.917	1.030	0.549-1.934
DQB1*04	0	7 (2.20)	0.356	0.917	0.411	0.023-7.312
DQB1*05	16 (32.00)	105 (33.02)	0.898	0.917	0.955	0.504-1.807
DQB1*06	8 (16.00)	46 (14.45)	0.756	0.917	1.126	0.497-2.552

[a]Fisher's Exact test; P^{adj}: P adjusted; OR: Odds Ratio; CI: Confidence interval.

significant earlier disease onset. A significant difference in the angiographic type of the disease between the HLA-B*52-positive and -negative TA patients was found. In addition, the presence of HLA-B*52 increased susceptibility to the more extensive disease form - angiographic type V. However, the presence of a different morphological type of the disease (aneurismal vs stenotic), as well as the presence of aortic regurgitation (the most frequent cardiovascular complication in our TA patients), did not differ significantly between patients who have and do not have HLA-B*52 allele.

The first-line treatment - oral prednisone or intravenous methylprednisolone was given to 23 (92%) patients. Additionally, methotrexate, azathioprine, mycophenolate mofetil, cyclophosphamide, and anti-tumor-necrosis factor-alpha monoclonal antibody, as the second-line treatment were given to 10 (40%), 3 (12%), 2 (8%), 1 (4%), and 1 (4%) patient, respectively.

TA patients with HLA-B*52 allele significantly more difficult reached a sustained clinical remission, despite the glucocorticoid therapy, and an additional immunosuppressant. Moreover, they required more frequently combined therapy of immunosuppressants with glucocorticoids. Neither severe vascular complications, nor the need for a vascular intervention were associated with the HLA-B*52 presence. We did not observe a statistically significant difference in the development of hypertension between the HLA-B*52 -positive and -negative patients. In addition, the HLA-B*52 positivity was not found to significantly affect inflammatory state (levels of inflammatory parameters and presence of systemic manifestations of inflammation) at the moment of the TA diagnosis

The frequencies of the two loci (DRB1*-DQB1*) haplotypes are shown in Table 4. However, statistical significance for the DRB1*15-DQB1*05 haplotype, that was initially found (P=0.039), was lost after the P value correction (P^{adj}=0.583).

Comprehensive data of all the tested HLA class I and the HLA class II loci in our TA cohort and the presence of the analyzed clinical characteristics have been shown in the Table 5.

Table 4. Frequencies of DRB1-DQB1* Haplotype in TA patients and controls

Haplotype	TA (n=50) Haplotype Frequency N (%)	Controls (n=318) Haplotype Frequency N (%)	P value[a]	P^{adj}	OR	CI (95%)
DRB1*01-DQB1*05	3 (6.00)	40 (12.89)	0.159	0.989	0.443	0.131-1.493
DRB1*03-DQB1*02	5 (10.00)	40 (12.89)	0.595	0.989	0.772	0.289-2.061
DRB1*04-DQB1*03	6 (12.00)	28 (8.81)	0.468	0.989	1.412	0.553-3.605
DRB1*07-DQB1*02	4 (8.00)	13 (4.09)	0.253	0.989	2.040	0.637-6.526
DRB1*07-DQB1*03	3 (6.00)	10 (3.14)	0.338	0.989	1.986	0.522-7.405
DRB1*08-DQB1*03	1 (2.00)	5 (1.57)	0.776	0.989	1.278	0.146-11.170
DRB1*08-DQB1*04	0	5 (1.57)	0.480	0.989	0.565	0.030-10.363
DRB1*10-DQB1*05	1 (2.00)	2 (0.63)	0.099	0.989	3.224	0.287-36.237
DRB1*11-DQB1*03	7 (14.00)	54 (16.98)	0.623	0.989	0.796	0.340-1.863
DRB1*12-DQB1*03	0	4 (1.26)	0.556	0.989	0.692	0.037-13.048
DRB1*13-DQB1*06	4 (8.00)	27 (8.49)	0.951	0.989	0.937	0.313-2.802
DRB1*14-DQB1*05	3 (6.00)	20 (6.29)	0.988	0.989	0.951	0.272-3.326
DRB1*15-DQB1*06	4 (8.00)	19 (5.97)	0.570	0.989	1.368	0.445-4.202
DRB1*15-DQB1*05	3 (6.00)	3 (0.94)	0.039	0.583	6.702	1.314-34.187
DRB1*16-DQB1*05	5 (10.00)	39 (12.26)	0.680	0.989	0.795	0.297-2.124

[a]Fisher's Exact test; Significant diference between TA patients and controls is bolded; P^{adj}: P adjusted; OR: Odds Ratio; CI: Confidence interval.

Table 5. HLA, demographic and clinical features of TA patients

	A1*	A2*	B1*	B2*	C1*	C2*	DRB1*	DRB1*	DQB1*	DQB1*	Age	Sex	AT	AR	R	INF	GC	IS	VI	VC	HT
1	A*02	A*29	B*07	B*15	C*03	C*15	DRB1*10	DRB1*14	DQB1*05	DQB1*05	37	f	IIb	1	2	+	+	+	-	-	-
2	A*03	A*24	B*44	B*52	C*02	C*12	DRB1*03	DRB1*15	DQB1*02	DQB1*06	26	f	V	2	3	+	+	+	-	-	-
3	A*26	A*68	B*35	B*39	C*04	C*12	DRB1*08	DRB1*16	DQB1*03	DQB1*05	63	f	V	1	1	+	+	-	-	-	+
4	A*02	A*26	B*50	B*58	C*03	C*06	DRB1*03	DRB1*07	DQB1*02	DQB1*02	45	f	I	0	0	-	-	-	-	+	+
5	A*02	A*02	B*15	B*51	C*03	C*16	DRB1*04	DRB1*16	DQB1*03	DQB1*05	27	f	IIb	1	1	+	+	-	-	-	-
6	A*11	A*32	B*35	B*55	C*03	C*04	DRB1*15	DRB1*16	DQB1*05	DQB1*06	53	f	IIb	0	0	+	-	-	+	-	+
7	A*23	A*32	B*18	B*57	C*12	C*18	DRB1*01	DRB1*15	DQB1*05	DQB1*06	63	f	IIa	1	3	+	+	+	-	-	-
8	A*24	A*26	B*57	B*58	C*03	C*06	DRB1*07	DRB1*11	DQB1*03	DQB1*03	53	f	V	0	1	+	+	+	+	+	+
9	A*02	A*02	B*18	B*44	C*05	C*07	DRB1*11	DRB1*13	DQB1*03	DQB1*06	63	f	IIb	0	1	+	+	-	-	-	+
10	A*32	A*32	B*39	B*40	C*02	C*12	DRB1*11	DRB1*11	DQB1*03	DQB1*03	55	f	IIa	1	1	+	+	-	-	-	+
11	A*02	A*24	B*44	B*52	C*05	C*12	DRB1*04	DRB1*15	DQB1*03	DQB1*05	42	f	V	1	2	+	+	+	-	-	-
12	A*02	A*68	B*18	B*49	C*05	C*07	DRB1*03	DRB1*07	DQB1*02	DQB1*02	65	f	IIb	1	0	-	-	+	+	-	-
13	A*01	A*32	B*08	B*40	C*02	C*07	DRB1*03	DRB1*16	DQB1*02	DQB1*05	57	m	V	1	1	+	+	-	-	-	+
14	A*03	A*24	B*18	B*35	C*04	C*07	DRB1*01	DRB1*11	DQB1*03	DQB1*05	59	f	IIb	1	1	+	+	-	-	-	-
15	A*02	A*11	B*35	B*51	C*04	C*16	DRB1*10	DRB1*11	DQB1*03	DQB1*05	50	f	IIa	0	0	+	+	-	+	+	-
16	A*24	A*25	B*50	B*57	C*06	C*06	DRB1*07	DRB1*07	DQB1*02	DQB1*03	40	f	V	1	2	+	+	+	-	+	-
17	A*01	A*24	B*40	B*52	C*12	C*15	DRB1*14	DRB1*15	DQB1*05	DQB1*05	24	f	V	1	2	+	+	+	-	+	+
18	A*02	A*11	B*35	B*52	C*04	C*12	DRB1*11	DRB1*15	DQB1*03	DQB1*05	16	f	III	0	3	+	+	+	-	+	-
19	A*02	A*11	B*39	B*52	C*12	C*12	DRB1*04	DRB1*15	DQB1*03	DQB1*06	15	f	V	1	3	+	+	+	-	-	-
20	A*02	A*03	B*15	B*51	C*03	C*14	DRB1*04	DRB1*14	DQB1*03	DQB1*05	27	f	V	1	3	+	+	+	-	-	+
21	A*24	A*24	B*15	B*15	C*03	C*03	DRB1*13	DRB1*16	DQB1*05	DQB1*06	55	f	IV	0	1	+	+	-	-	-	+
22	A*01	A*11	B*08	B*51	C*07	C*15	DRB1*03	DRB1*04	DQB1*02	DQB1*03	53	f	IIa	1	1	+	+	-	-	-	-
23	A*30	A*32	B*13	B*27	C*02	C*06	DRB1*07	DRB1*13	DQB1*02	DQB1*06	36	f	IIb	1	1	+	+	-	-	-	+
24	A*03	A*26	B1*38	B*57	C*06	C*12	DRB1*07	DRB1*13	DQB1*03	DQB1*06	59	m	V	1	1	+	+	-	+	+	-
25	A*25	A*32	B*15	B*51	C*01	C*03	DRB1*01	DRB1*04	DQB1*03	DQB1*05	34	f	V	1	3	+	+	+	+	-	-

HLA*52 -carriers are bolded; AT: Angiographic type; AR: Aortic regurgitation: 0 - not present, 1 - mild to moderate, 2- severe; R: Remission achieved: 0-no therapy, 1-on glucocorticoids (gcs), 2- additional immunosuppressive agents along with gcs, 3- no remission on treatment; INF: Active inflammation on appearance; GC: Treatment with glucocorticoids; IS: Additional immunosuppressive agents; VI: Vascular intervention - surgery or endovascular procedure; VC: Vascular complications; presence of any of listed: ischemic heart disease, cerebrovascular insult, critical limb ischemia; HT – Hypertension.

DISCUSSION

This study was conducted to provide, for the first time, a comprehensive analysis of the five loci polymorphisms (HLA-A*, HLA-B*, HLA-C*, HLA-DRB1*, and HLA-DQB1*) in the TA patients in Serbia. We tested samples for the presence of the 58 HLA class I, and the 18 HLA class II allelic groups, that were previously described in the Serbian healthy population (22). The loci HLA-A*, HLA-B*, HLA-DRB1*, and HLA-DQB1* in the TA patients deviated from the H-W (P=2.2e-05; P=5.7e-05; P=2.2e-16; and P=4e-06, respectively), while the analysis of the H-W in the control group confirmed the equilibrium state for all loci tested (22). We assume a small sample size of the TA patients and a possible selection bias might have contributed to the H-W disequilibrium.

Genetic studies performed across different countries and ethnicities have revealed that some HLA alleles might contribute to higher susceptibility to the TA. The strongest association of the disease was found with the HLA-B*52 allelic group, and very few studies reported no association between the HLA-B*52 and the TA (25).

HLA polymorphisms in the TA were studied worldwide, starting from the late eighties, when Isohisa et al. identified Bw52 in 43,9%, and Naito et al. found B5 allelic group to be positive in 66% of Japanese TA patients (26, 27). It is very important to note that the frequency of the HLA-B52 differs among ethnic groups. Kimura et al. reported its variation in the healthy controls as follows: 24.2% in Japan, 13.2% in India, 7.2% in Mexico, 6.1% in Thailand, 6.0% in the USA, 5.3% in Italy, 4.9% in Korea, 4.2% in China (17). It was detected in 3,5% of the Turkey healthy controls (28), and even lower in Balkan populations: 2,6% in the Macedonians and the Greeks of the sub-Saharan origin (29), and 2,1% of Romanian (30). The frequency of the HLA-B*52 allele in the Serbian healthy population is 1.2% (22). Keeping that in mind, it is not surprising that a wide range of the HLA-B*52 frequencies in ethnically divergent TA cohorts have been reported.

Previously found a positive association of the TA with the HLA-B39.2 in Japan (17) however, was not confirmed in recent studies (6, 31). The difference between the results of these studies was explained by the prevalence of B39 antigen (B*3901) in the controls (31). Also, the HLA-B*67, which is found to be strongly associated with the TA in Japan (15) was absent in both, experimental and control cohorts in Serbia, which is in line with the consideration that the HLA-B*67 allelic group is a characteristic of the East Asian people.

In Mexicans, the TA was associated with the HLA-DR6 (DRB1*1301) (32), -B*39, -B*15, and -B*40 (11). A study of Korean patients revealed A*3001, B*5201, and DRB1*1502 as susceptibility alleles, while A*2602 might be a protective allele in the TA pathogenesis (8). However, none of the alleles described to exhibit direct preventive effect in particular populations appeared to be beneficial globally, nor found to be associated with the TA in our cohort.

Very few data about the HLA polymorphisms in European Caucasian TA patients have been published so far. In 2009, Karageorgaki TZ et al. analysed epidemiological, clinical, and immunogenetic features in the Greek TA cohort. They found the HLA-B52 expression in 38% (7/19) of the patients, and it was significantly higher than in the healthy controls (2,6%; 6/246), whilst the significance was lost for the HLA-DRB1*1502 and the HLA-DQB1*0601 after the p-value correction (12). The frequency of the HLA-B*52 allelic group in our patients is comparable to those in Greece. Neither the HLA-DRB1*15 nor the HLA-DQB1*06 was connected with an increased risk for the TA patients in Serbia.

Interestingly, despite the well-known high LD between DRB1*15 and DQB1*06 alleles, in our TA patients, the HLA-DRB1*15 was also found to be linked to the -DQB1*05,

making a haplotype DRB1*15-DQB1*05, which is rarely found in the Serbian healthy population (22). The haplotype DRB1*15-DQB1*05 was present in 6% of TA patients, and it was significantly more frequent than in the healthy controls (0.94%). However, statistical significance found between frequency of the DRB1*15-DQB1*05 haplotype in the TA patients and the controls (P=0.039), was lost after the p-value adjustment (p^{adj}=0.583) (Table 4). Even more interestingly, the haplotype DRB1*15-DQB1*05 was exclusively present in patients that had the HLA B*52, which may raise a question of the possible importance of the B*52-DRB1*15-DQB1*05 haplotype in the TA pathogenesis (Table 1).

Several studies that assessed the associations of the class I HLA and the TA have revealed susceptibility allele HLA-Cw*12:02 in Japanese, Turkish, and European-American patients (13, 31). It is considered to be dependent on HLA-B*52, due to high LD, and probably, represents the same genetic effect (4). Although the observed frequencies for other tested alleles (HLA-C*12 and -DRB1*15) in the TA patients in Serbia were not significantly higher than expected, it is an interesting observation that all of our HLA-B*52 positive patients, were carriers of the HLA-C*12 and -DRB1*15 alleles, suggesting possible importance of such allelic combination in the disease development.

By contrasting the susceptibility alleles, in 32% of patients (8/25), we determined the HLA-C*03, which might have a protective role. All of the patients except one, having a -C*03 group of allele exhibited clinically milder forms of TA, reaching and maintaining a long-term clinical remission without a need for additional immunosuppressive medication.

Although this study represents the first comprehensive HLA analysis in the TA patients in Serbia, there are some drawbacks to this strategy. Patients were recruited from the large University Clinical Center, which is considered a center for rare diseases within the whole country, and in line with this, it is a place where the most severe cases of the diseases are supposed to be diagnosed and treated. A milder form of the TA might be followed and treated in smaller medical centers within the country, which might have resulted in selection bias. On the other hand, it serves as the referral center for the whole country, so we also recruited the TA patients who came for a diagnostic reason at another clinic among the University Clinical Center: Vascular, Cardiology, Radiology, Neurology, Clinic of Infective Disease, NMR, Radiology and PET Center. Additionally, as limitations were considered: the estimated power of the study calculation of 0.5 and the unequal number of persons tested for all the five HLA loci within the control groups (HLA-C*and HLA-DQB1 were available for 159 controls).

CONCLUSION

Our findings indicate that the HLA-B*52 allele contributes to higher susceptibility to the TA. Moreover, carriage of the HLA-B*52 is associated with significantly earlier disease onset, more severe clinical presentations, and a poorer response to the first-line treatment. The association of the HLA-A*32, the HLA-B*15, and the HLA-B*57 allelic groups, and the DRB1*15:02-DQB1*05 haplotype with the TA, as the genetic susceptibility factors, still need to be confirmed. Our study has shown, to our knowledge, for the first time, that the HLA-C*03 could have a protective role in the TA pathogenesis. Additional research with a bigger sample size would be beneficial to better evaluate the potential roles of these alleles in the TA development.

Conflicts of Interest: None declared.

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