
HLA alleles in Korean patients with Takayasu arteritis

S.-W. Lee¹, O.-J. Kwon², M.-C. Park¹, H.-B. Oh³, Y.-B. Park¹, S.-K. Lee¹

¹Division of Rheumatology, Department of Internal Medicine, Institute for Immunology and Immunological Diseases, Brain Korea 21 Project for Medical Science, Graduate School of Yonsei University, Yonsei University College of Medicine; ²Biosewom Institute of Bioscience and Biotechnology, ³Department of Laboratory Medicine, University of Ulsan College of Medicine, Seoul, Korea.

Sang-Won Lee¹, MD, Oh-Joong Kwon², PhD, Min-Chan Park¹, MD, Heung-Bum Oh³, MD, PhD, Yong-Beom Park¹, MD, PhD, Soo-Kon Lee¹, MD, PhD.

This work was supported by the Korea Research Foundation Grant (KRF-2003-E00107).

Please address correspondence and reprint requests to: Yong-Beom Park, MD, PhD, Department of Internal Medicine, Yonsei University College of Medicine, 134 Shinchon-dong, Seodaemun-ku, Seoul, Korea, 120-752.

E-mail: yongbepark@yumc.yonsei.ac.kr

Received on September 8, 2006; accepted in revised form on February 1, 2007.

Clin Exp Rheumatol 2007; 25 (Suppl. 44): S18-S22.

© Copyright CLINICAL AND EXPERIMENTAL RHEUMATOLOGY 2007.

Key words: Human leukocyte antigen (HLA), susceptibility, Takayasu arteritis.

ABSTRACT

*In this study, we investigated the HLA allele and haplotype frequencies, and the association of HLA alleles with serious complications and angiographic findings in Korean patients with Takayasu arteritis (TA) compared with healthy controls. Sixty-one patients (56 women, 5 men), diagnosed with TA between January 1995 and December 2005, were studied. Ninety-five healthy men and women were selected as controls. Clinical manifestations were assessed and angiographies were performed at the time of diagnosis in all TA patients. Genotypes of the HLA-A, -B and -DRB1 loci were determined using the polymerase chain reaction-sequencing-based typing (PCR-SBT) method. The mean age at the time of diagnosis of TA was 37.0 ± 12.1 years. Compared with controls, the frequencies of A*3001 ($p = 0.048$), B*5201 ($p = 0.025$), and DRB1*1502 ($p = 0.046$) alleles were significantly higher in TA patients, and the frequency of A*2602 was significantly lower in TA patients when compared with controls ($p = 0.047$). The haplotype containing A*2402-B*5201-DRB1*1502 was significantly increased in TA patients ($\chi^2 = 5.45$, $p = 0.01$). Further, among the serious complication of TA, congestive heart failure (CHF) was found to be associated with B*5201 (OR = 5.94, $p < 0.05$, 95% CI = 1.04~33.85). These data suggest that A*3001, B*5201, and DRB1*1502 alleles might increase the susceptibility to TA, while A*2602 might protect against TA. Further, our results reveal that the haplotype A*2402-B*5201-DRB1*1502 could be a risk factor for TA, and the allele B*5201 is significantly associated with CHF.*

Introduction

Takayasu's arteritis (TA) is a granulomatous vasculitis of unknown etiology

that affects the aorta, its major branches and the pulmonary arteries (1). The incidence of TA varies according to geographical and ethnic background. TA is found in Asian and Latin American countries more frequently than in European and North American countries (2). The reported clinical manifestations of TA vary between countries and ethnic groups (3, 4). In addition, these observations suggest a strong association between TA and genetic background. Human leukocyte antigen (HLA) has been considered a candidate for a genetic factor determining the susceptibility to TA, because it has a role in regulating immune responses (5). Moreover, HLA alleles which are highly polymorphic show ethnic variation in frequency. This could explain the geographical and ethnic variation associated with the incidence of TA. The HLA alleles B*5201, B*3902, and DRB1*1502 were reported to be associated with TA in Japan (6-8), A31 and B52 in Thailand (9), B5 and its subtypes B51 and B52 in India (10), DRB1*1602 and DRB1*1001 in Colombian Mestizo patients (11), B5, B15, B52 and DR6 in Mexican Mestizo patients (12), and A2, A9, B35 and DR7 in Arabs (13). It was also reported that genetic factors seem to be implicated not only in the susceptibility, but also in the severity and outcome of TA (14). It is, therefore, important to investigate HLA alleles specific to different human populations in order to predict the development and understand the pathophysiology of TA. An association of HLA-Bw52 and B5 with TA in Korean TA patients was reported (15, 16), but there has been no report on the allelic level based on high resolution typing in Korean TA patients. In this study, we investigated the association of the HLA alleles and haplotypes with serious complications and angiographic findings in patients with TA.

Table I. Clinical manifestations and angiographic findings in patients with Takayasu arteritis.

	Patients (N)	Frequency (%)
Sex, men/women	5 / 56	8.2 / 91.8
Age at diagnosis		
≤ 40 years old	41	67.2
> 40 years old	20	32.8
Systemic symptoms		
Fever	3	4.9
General weakness	26	42.6
Fatigue	23	37.7
Weight loss	6	9.8
Headache	33	54.1
Nausea	6	9.8
Vomiting	2	3.3
Numbness	5	8.2
Vascular manifestations		
Vascular bruit	36	59.0
Systolic blood pressure limb difference	49	80.3
Claudication	18	29.5
Pulse anomaly	33	54.1
Hypertension	32	52.5
Serious complications		
Congestive heart failure ^a	21	34.4
Ischemic heart disease	17	27.9
Cerebrovascular accident	11	18.0
Valvular heart disease	8	13.1
Retinopathy	5	8.2
Angiographic findings		
Type		
I	19	31.2
IIa	8	13.1
IIb	3	4.9
III	6	9.8
IV	7	11.5
V	18	29.5

^aCongestive heart failure was evaluated by Echocardiography with tissue Doppler imaging.

Materials and methods

Patients

Sixty-one patients (56 women, 5 men), diagnosed with TA between January 1995 and December 2004, were studied. They all fulfilled the American College of Rheumatology 1990 criteria for the classification of TA (17). The mean age of the patients at the time of diagnosis with TA was 37.0 ± 12.1 years (range 14 to 64 years). Ninety-five healthy men and women were selected as controls. Patient consent and ethical approval were obtained for this study.

Clinical manifestations and angiographic findings

Clinical manifestations were assessed

and angiographies were performed at the time of diagnosis in all patients with TA. A life threatening or disabling condition attributed to TA was defined as a serious complication, including cerebrovascular accident (CVA), congestive heart failure (CHF), valvular heart disease (VHD), ischemic heart disease (IHD), and retinopathy (18). Angiographic findings were classified according to the International Takayasu's Arteritis Conference of 1994 (19). Types of arteriogram are as follows: type I, involvement of the main branches from the aortic arch; type IIa, involvement of the ascending aorta, aortic arch and its branches; type IIb, involvement of the ascending aorta, aortic arch and its

branches, thoracic descending aorta; type III, involvement of the thoracic descending aorta, abdominal aorta, and/or renal arteries; type IV, involvement of the abdominal aorta and/or renal arteries; and type V, the combined features of type IIb and IV.

High-resolution HLA genotyping

Whole blood was collected from 61 TA patients and 95 healthy controls, and stored at -20°C until use. DNA was extracted from the blood samples using the LaboPassTM Blood mini Kit (Cosmogenetech, Seoul, Korea). In short, the whole blood sample was mixed with 20 μl of proteinase K and 200 μl of BL solution, incubated at 56 μl for 10 minutes, transferred to a column with 200 μl of ethanol, and then centrifuged at 10,000 rpm for 1 minute. The column was then washed with 300 μl BW solution, and treated with 500 μl NW solution. Genomic DNA was eluted with 200 μl tertiary distilled water, and used as the template for polymerase chain reaction (PCR).

High resolution HLA genotyping was performed. Alleles of the HLA-A, -B, and -DRB1 loci were genotyped using the PCR-sequencing-based typing (PCR-SBT) kit (Biosewoom, Inc., Seoul, Korea). The genes of HLA-A, -B, and -DRB1 were amplified by PCR. For SBT, exon 2 and exon 3 of both HLA-A and -B, and exon 2 of HLA-DRB1 were sequenced directly. Sequencing was performed using the BigDyeTM Terminator v3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA) and purified by the WizardMagneSilTM Sequencing Reaction Clean-Up System (Promega, CA, USA). The purified products were electrophoresed on an ABI PRISM[®] 3730xl Genetic Analyzer (Applied Biosystems, Foster City, CA). Sequences were analyzed with the HLA analysis program (Biosewoom, Inc., Seoul, Korea).

Statistical analysis

The SAS/genetic module in SAS version 9.1 (SAS Institute Inc., NC, USA) was used for statistical analysis. The allele frequencies of HLA-A, -B, and -DRB1 in the patient and control groups were compared using the chi-square

Table II. HLA DNA-types in patients with Takayasu arteritis.

HLA alleles	Patients (N = 61) N(%)	Controls (N = 95) N(%)	<i>p</i> value	HLA alleles	Patients (N = 61) N(%)	Controls (N = 95) N(%)	<i>p</i> value
A*0101	2 (1.6)	4 (2.1)	NS ^a	B*4403	7 (5.7)	17 (8.9)	NS
A*0201	25 (20.5)	29 (15.3)	NS	B*4601	3 (2.5)	11 (5.8)	NS
A*0203	0 (0)	1 (0.5)	NS	B*4801	3 (2.5)	6 (3.2)	NS
A*0205	0 (0)	1 (0.5)	NS	B*5001	0 (0)	1 (0.5)	NS
A*0206	11 (9.0)	12 (6.3)	NS	B*5101	7 (5.7)	21 (11.1)	NS
A*0207	4 (3.3)	7 (3.7)	NS	B*5102	0 (0)	1 (0.5)	NS
A*0215	0 (0)	1 (0.5)	NS	B*5201	10 (8.2)	5 (2.6)	0.025
A*0301	2 (1.6)	2 (1.1)	NS	B*5401	9 (7.4)	10 (5.3)	NS
A*0302	3 (2.5)	0 (0)	0.030	B*5502	4 (3.3)	5 (2.6)	NS
A*1101	13 (10.7)	22 (11.6)	NS	B*5601	1 (0.8)	1 (0.5)	NS
A*1102	1 (0.8)	0 (0)	NS	B*5701	1 (0.8)	0 (0)	NS
A*2402	23 (18.9)	41 (21.6)	NS	B*5801	6 (4.9)	12 (6.3)	NS
A*2408	1 (0.8)	1 (0.5)	NS	B*5901	1 (0.8)	3 (1.6)	NS
A*2420	1 (0.8)	1 (0.5)	NS	B*6701	0 (0)	4 (2.1)	NS
A*2601	4 (3.3)	10 (5.3)	NS				
A*2602	0 (0)	6 (3.2)	0.047	DRB1*0101	10 (8.2)	13 (6.8)	NS
A*2603	1 (0.8)	0 (0)	NS	DRB1*0301	4 (3.3)	7 (3.7)	NS
A*2610	0 (0)	1 (0.5)	NS	DRB1*0403	2 (1.6)	3 (1.6)	NS
A*2910	0 (0)	1 (0.5)	NS	DRB1*0404	2 (1.6)	6 (3.2)	NS
A*3001	9 (7.4)	5 (2.6)	0.048	DRB1*0405	8 (6.6)	12 (6.3)	NS
A*3004	1 (0.8)	3 (1.6)	NS	DRB1*0406	3 (2.5)	11 (5.8)	NS
A*3101	8 (6.6)	10 (5.3)	NS	DRB1*0407	0 (0)	1 (0.5)	NS
A*3201	1 (0.8)	0 (0)	NS	DRB1*0408	0 (0)	1 (0.5)	NS
A*3303	12 (9.8)	32 (16.8)	NS	DRB1*0410	0 (0)	5 (2.6)	NS
				DRB1*0451	1 (0.8)	0 (0)	NS
B*0702	3 (2.5)	6 (3.2)	NS	DRB1*0701	13 (10.7)	12 (6.3)	NS
B*0705/6	0 (0)	1 (0.5)	NS	DRB1*0801	2 (1.6)	0 (0)	NS
B*0801	3 (2.5)	2 (1.1)	NS	DRB1*0802	4 (3.3)	3 (1.6)	NS
B*1301	2 (1.6)	5 (2.6)	NS	DRB1*0803	9 (7.4)	14 (7.4)	NS
B*1302	10 (8.2)	8 (4.2)	NS	DRB1*0901	12 (9.8)	19 (10.0)	NS
B*1401	1 (0.8)	3 (1.6)	NS	DRB1*1001	4 (3.3)	1 (0.5)	NS
B*1501	13 (10.7)	16 (8.4)	NS	DRB1*1101	5 (4.1)	9 (4.7)	NS
B*1507	0 (0)	1 (0.5)	NS	DRB1*1106	1 (0.8)	0 (0)	NS
B*1511	2 (1.6)	0 (0)	NS	DRB1*1201	5 (4.1)	8 (4.2)	NS
B*1518	1 (0.8)	1 (0.5)	NS	DRB1*1202	3 (2.5)	9 (4.7)	NS
B*1538	0 (0)	1 (0.5)	NS	DRB1*1301	1 (0.8)	6 (3.2)	NS
B*2705	7 (5.7)	8 (4.2)	NS	DRB1*1302	6 (4.9)	17 (8.9)	NS
B*3501	6 (4.9)	15 (7.9)	NS	DRB1*1401	4 (3.3)	5 (2.6)	NS
B*3511	0 (0)	1 (0.5)	NS	DRB1*1402	1 (0.8)	0 (0)	NS
B*3701	2 (1.6)	2 (1.1)	NS	DRB1*1403	0 (0)	1 (0.5)	NS
B*3802	0 (0)	1 (0.5)	NS	DRB1*1405	3 (2.5)	5 (2.6)	NS
B*3901	1 (0.8)	4 (2.1)	NS	DRB1*1407	0 (0)	2 (1.1)	NS
B*4001	7 (5.7)	6 (3.2)	NS	DRB1*1410	3 (2.5)	0 (0)	0.030
B*4002	7 (5.7)	5 (2.6)	NS	DRB1*1412	0 (0)	1 (0.5)	NS
B*4003	1 (0.8)	1 (0.5)	NS	DRB1*1501	8 (6.6)	14 (7.4)	NS
B*4006	3 (2.5)	5 (2.6)	NS	DRB1*1502	8 (6.6)	4 (2.1)	0.046
B*4402	1 (0.8)	1 (0.5)	NS	DRB1*1602	0 (0)	1 (0.5)	NS

NS: not significant.

statistics. The haplotype procedure in the program was used to compare the haplotype frequency between patients and controls. Low-frequency alleles

(< 3%) and haplotypes (< 2%) both in the patient and control group were not analyzed in order to reduce the number of comparisons with inadequate power.

Odds ratio (OR) was assessed using binary logistic regression test in SPSS package for Windows version 11.5. The level of significance was set at 0.05.

Results

Patients' characteristics

Forty-one patients (67.2%) were less than 40 years old when diagnosed with TA. The most common systemic symptom was headache (54.1%), followed by general weakness (42.6%), and fatigue (37.7%). A blood pressure difference in either upper or lower extremities (80.3%) was the most frequent vascular manifestations. Hypertension was found in 32 patients (52.5%). Nineteen of 61 patients had angiographic type I (31.1%), 8 type IIa (13.1%), 3 type IIb (4.9%), 6 type III (9.8%), 7 type IV (11.5%), and 18 type V (29.5%).

Thirty-eight patients (62.3%) had a total of 62 serious complications. CHF was found in 21 patients (34.4%). IHD, CVA, VHD, and retinopathy were found in 17 (27.9%), 11 (18.0%), 8 (13.1%), and 5 (8.2%) patients, respectively. Table I shows the demographic features, clinical manifestations, and angiographic types at the time of diagnosis of TA.

HLA alleles in patients with TA

Compared with the HLA allele frequencies in controls, the frequencies of A*3001 ($p = 0.048$), B*5201 ($p = 0.025$), and DRB1*1502 ($p = 0.046$) alleles were significantly higher in TA patients. However, the frequency of A*2602 allele in TA patients was significantly lower than in controls ($p = 0.047$) (Table II). Seven out of 8 TA patients with DRB1*1502 allele also had B*5201 allele.

Haplotype frequency in patients with TA

Only one haplotype, A*2402-B*5201-DRB1*1502 (5.7% vs. 1.6%), showed significant difference between patients and healthy controls ($\chi^2=5.45$, $p = 0.01$). A*3001 containing haplotype, A*3001-B*1302-DRB1*0701, was also increased in patient group (7.4% vs. 2.6%), but not statistically significant. Other haplotypes did not exhibit any significant difference between the two groups.

Association of HLA alleles with serious complications and angiographic findings

We investigated the association between HLA alleles and serious com-

plications and angiographic findings. Since DRB1*1502 was highly linked to B*5201, we divided TA patients into 3 groups according to whether they have A*3001, B*5201, or not. Six patients were A*3001(+) and B*5201(-), 7 patients A*3001(-) and B*5201(+), and 45 patients A*3001(-) and B*5201(-). Three TA patients with A*3001(+) and B*5201(+) were excluded to evaluate the independent effect of each HLA allele on clinical manifestations. CVA, CHF, VHD, IHD, and retinopathy and angiographic types were analyzed and compared among the 3 groups. The frequency of CHF in the A*3001(-) and B*5201(+) group was significantly increased in comparison with the other two groups. Other serious complications and angiographic findings exhibited no significant association with differences in allele frequencies among the 3 groups. CHF was observed significantly more frequently in TA patients with B*5201 than in those without this allele (OR = 5.94, $p < 0.05$, 95% CI = 1.04~33.85).

Discussion

Our data suggest that A*3001, B*5201, and DRB1*1502 alleles are associated with the disease. B52 and DRB1*1502 alleles were reported to be associated with Japanese patients (8), however, A*3001 allele has not previously been reported. The B*3902 allele, which was reported to be associated with TA in Japanese patients (6), showed no significant association in our patient cohort.

By contrast, several HLA alleles have been reported to be less frequent in TA patients than in healthy controls; DRB1*0405 was reported to be negatively associated with TA in Japanese patients (5), and DR1 in North American ones (20). It is noteworthy that the A*2602 allele was not detected in 6 healthy controls in this study. The A*2602 allele was, therefore, inversely associated with TA, suggesting that either A*2602 itself might control susceptibility to TA, thereby providing protection against the development of TA in the general population.

Linkage disequilibrium results in a

stronger association than expected between HLA alleles within a haplotype. Therefore, the enhanced possibility of a haplotype including HLA alleles associated with susceptibility to TA could increase the incidence of the disease. In this study, seven TA patients shared both the B*5201 and DRB1*1502 alleles, and this observation suggests that there might be a strong linkage-disequilibrium between the two alleles in the Korean population, similarly to the Japanese population (21). In this study, one haplotype containing A*2402, B*5201, and DRB1*1502 showed a significant frequency difference between patients with TA and healthy controls. Therefore, this haplotype could be postulated as a risk factor for development of TA.

The genetic association of serious complications with a specific HLA allele or haplotype has been reported. Pulmonary and aortic valve disorders were observed more frequently in TA patients with the B52-Dw12 haplotype (21, 22). In this study, CHF was observed significantly more frequent in TA patients with B*5201 than in those without. This suggests that there is a significant association between the allele B*5201 and the presence of CHF in patients with TA.

In conclusion, our data suggest that A*3001, B*5201, and DRB1*1502 alleles might increase the susceptibility to TA, while A*2602 might protect against TA. Further, our results reveal that the haplotype A*2402-B*5201-DRB1*1502 could be a risk factor for TA, and the allele B*5201 is significantly associated with CHF in Korean patients.

References

- JENNETTE JC, FALK RJ, ANDRASSY K *et al.*: Nomenclature of systemic vasculitides. Proposal of an international consensus conference. *Arthritis Rheum* 1994; 37:187-92.
- KERR GS, HALLAHAN CW, GIORDANO J *et al.*: Takayasu arteritis. *Ann Intern Med* 1994; 120: 919-29.
- GONZALEZ-GAY MA, GARCIA-PORRUA C: Epidemiology of the vasculitides. *Rheum Dis Clin North Am* 2001; 27: 729-49.
- NUMANO F: Differences in clinical presentation and outcome in different countries for Takayasu's arteritis. *Curr Opin Rheumatol* 1997; 9: 12-5.
- WEYAND CM, GORONZY JJ: Molecular ap-

- proaches toward pathologic mechanisms in giant cell arteritis and Takayasu's arteritis. *Curr Opin Rheumatol* 1995; 7: 30-6.
6. KIMURA A, KITAMURA H, DATE Y, NUMANO F: Comprehensive analysis of HLA genes in Takayasu arteritis in Japan. *Int J Cardiol* 1996; 54 (Suppl): S61-9.
 7. YOSHIDA M, KIMURA A, KATSURAGI K, NUMANO F, SASAZUKI T: DNA typing of HLA-B gene in Takayasu's arteritis. *Tissue Antigens* 1993; 42: 87-90.
 8. DONG RP, KIMURA A, NUMANO F *et al.*: HLA-DP antigen and Takayasu arteritis. *Tissue Antigens* 1992; 39: 106-10.
 9. CHAROENWONGSE P, KANGWANSHIRATADA O, BOONNAM R, HOOMSINDHU U: The association between the HLA antigens and Takayasu's arteritis in Thai patients. *Int J Cardiol* 1998; 66 (Suppl. 1): S117-20.
 10. MEHRA NK, JAINI R, BALAMURUGAN A *et al.*: Immunogenetic analysis of Takayasu arteritis in Indian patients. *Int J Cardiol* 1998; 66 (Suppl. 11): S127-32.
 11. SALAZAR M, VARELA A, RAMIREZ LA *et al.*: Association of HLA-DRB1*1602 and DRB1*1001 with Takayasu arteritis in Colombian mestizos as markers of Amerindian ancestry. *Int J Cardiol* 2000; 75 (Suppl. 1): S113-6.
 12. VARGAS-ALARCON G, FLORES-DOMINGUEZ C, HERNANDEZ-PACHECO G *et al.*: Immunogenetics and clinical aspects of Takayasu's arteritis patients in a Mexican Mestizo population. *Clin Exp Rheumatol* 2001; 19: 439-43.
 13. FLORES-DOMINGUEZ C, HERNANDEZ-PACHECO G, ZUNIGA J *et al.*: Alleles of the major histocompatibility system associated with susceptibility to the development of Takayasu's arteritis. *Gac Med Mex* 2002; 138: 177-83.
 14. FIETTA P: Systemic vasculitides: immunogenetics and familial clustering. *Clin Exp Rheumatol* 2004; 22: 238-51.
 15. PARK MH, PARK YB: HLA typing of Takayasu arteritis in Korea. *Heart Vessels Suppl* 1992; 7: 81-4.
 16. YAJIMA M, NUMANO F, PARK YB, SAGAR S: Comparative studies of patients with Takayasu arteritis in Japan, Korea and India—comparison of clinical manifestations, angiography and HLA-B antigen. *Jpn Circ J* 1994; 58: 9-14.
 17. AREND WP, MICHEL BA, BLOCH DA *et al.*: The American College of Rheumatology 1990 criteria for the classification of Takayasu arteritis. *Arthritis Rheum* 1990; 33: 1129-34.
 18. PARK MC, LEE SW, PARK YB, CHUNG NS, LEE SK: Clinical characteristics and outcomes of Takayasu's arteritis: analysis of 108 patients using standardized criteria for diagnosis, activity assessment, and angiographic classification. *Scand J Rheumatol* 2005; 34: 284-92.
 19. HATA A, NODA M, MORIWAKI R, NUMANO F: Angiographic findings of Takayasu arteritis: new classification. *Int J Cardiol* 1996; 54 (Suppl.): S155-63.
 20. KHRAISHI MM, GLADMAN DD, DAGENAIS P, FAM AG, KEYSTONE EC: HLA antigens in North American patients with Takayasu arteritis. *Arthritis Rheum* 1992; 35: 573-5.
 21. KITAMURA H, KOBAYASHI Y, KIMURA A, NUMANO F: Association of clinical manifestations with HLA-B alleles in Takayasu arteritis. *Int J Cardiol* 1998; 66 (Suppl. 1): S121-6.
 22. MORIWAKI R, NUMANO F: Takayasu arteritis: follow-up studies for 20 years. *Heart Vessels Suppl* 1992; 7: 138-45.