Tohoku J. Exp. Med., 2016, **239**, 67-72

The Severity of Takayasu Arteritis Is Associated with the HLA-B52 Allele in Japanese Patients

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Takayasu arteritis (TA) is a type of vasculitis that affects the large elastic arteries, specifically the aorta and its main branches. It has been reported that TA occurred most frequently in Nagasaki Prefecture, the western area in Japan. We retrospectively collected the information of 34 patients with TA, diagnosed using the American College of Rheumatology 1990 criteria for the classification of TA, from the medical records of Nagasaki University Hospital from 2003 to 2015, and we investigated the clinical characteristics of these TA patients. Among the 35 patients, 25 patients were examined for the existence of the HLA-B52 allele that has been reported to influence TA susceptibility. Seventeen patients (68.0%) of the 25 patients were HLA-B52-allele-positive, which was defined as the state of having at least one HLA-B52 allele. There was a significant difference in the rate of smokers: HLA-B52-allele-positive: six patients (35.3%) vs. HLA-B52-allele-negative: 0 (0.0%). The C-reactive protein level in the HLA-B52-positive patients (9.0 ± 6.4 mg/dL) was significantly higher than that in the HLA-B52-negative patients (3.2 ± 3.9 mg/dL). All HLA-B52-allele-positive patients were found to be active according to Kerr's criteria. The HLA-B52-positive patients' initial prednisolone dosage (37.7 ± 8.6 mg/day) was significantly higher than that of the HLA-B52-allele-negative patients (23.1 ± 13.1 mg/day). Thus, the HLA-B52 allele is associated with the disease activity and the steroid requirements of TA patients. Furthermore, our present findings have revealed for the first time that the HLA-B52 allele and smoking might be associated with the onset of TA.

Keywords: disease activity; HLA-B52; Japanese; smoking; Takayasu arteritis
Tohoku J. Exp. Med., 2016 May, **239** (1), 67-72.

Introduction

Takayasu arteritis (TA) is a type of vasculitis of the large elastic arteries, specifically the aorta and its main branches (Weyand and Goronzy 2001). TA is a rare disease that affects primarily adolescent girls and young women. TA can occur in all races and geographic regions, but an international survey among 20 countries has indicated differences in the clinical spectrum of TA in different ethnic groups, and the incidence of TA in Japan is the highest. The survey of the Research Committee on Intractable Vasculitis Syndrome requested by the Ministry of Health, Labor and Welfare of Japan indicated that TA occurred most frequently in Nagasaki Prefecture, the westernmost area in Japan (Tanemoto et al. 2013).

The etiology of TA is still unknown, but recent studies have indicated that some genetic factors are involved in the TA disease process. One of the susceptible genes is human leukocyte antigen (HLA)-B52 allele (Sahin et al. 2012). A comprehensive analysis of HLA genes in TA patients revealed a strong association between TA and B52, which is located in the peptide-binding groove of the HLA-B molecule (Silver et al. 1992).

It is interesting to note that the distribution of HLA-B52 antigens differs among ethnic groups (Kimura et al. 1996). They reported that the frequencies of HLA-B52 allele in healthy control subjects were as follows: Japan 24.2%, India 13.2%, Mexico 7.2%, Thailand 6.1%, USA 6.0%, Italy 5.3%, Korea 4.9% and China 4.2%. Tokunaga et al. (1996, 1997) examined the frequencies of HLA-B52-DR2 in Japan and the east Asian countries. They reported that the frequency of HLA-B52-DR2 (8.6%) was the highest in healthy Japanese individuals. Moreover, HLA-B52-DR2 was detected in 13.5% of the population of

Received January 20, 2016; revised and accepted April 26, 2016. Published online May 18, 2016; doi: 10.1620/tjem.239.67.
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Nagasaki Prefecture and neighboring areas, which was the highest in Japan. The ethnic differences in the distribution of the HLA-B52 antigens may be the basis of the ethnic differences in the prevalence of TA.

However, the role of the HLA-B52 antigen in TA has not yet been completely defined. Over 20 years ago it was reported that features of a more severe and refractory disease are also associated with the presence of HLA-B52 in Japan: higher blood pressure, acute phase response, and a need for corticosteroids (Numano et al. 1982; Moriwaki and Numano 1992). However, Takamura et al. (2012) subsequently reported that no significant differences were observed between HLA-B52-positive patients and HLA-B52-negative patients in the following clinical characteristics: age at disease onset, distribution of arteritis, pulmonary involvement, aortic regurgitation (AR), systemic hypertension, steroid resistance, and recurrence rate. Kitamura et al. (1998) reported that the frequencies of AR, ischemic heart disease (IHD), and pulmonary infarction in the HLA-B52-positive group were significantly increased compared to those in the HLA-B52-negative group. The association between HLA-B52 and the activity of TA is controversial.

In the present study we examined the frequency of HLA-B52 allele in patients with TA in west Japan population with high HLA-B52 allele frequency, and we investigated the characteristics in HLA-B52-positive TA patients.

Patients and Methods

Patients

We performed a retrospective study of a series of TA patients diagnosed using the American College of Rheumatology (ACR) classification criteria (1990). These criteria include age at disease onset ≥ 40 years, claudication of extremities, decreased brachial artery pulse, blood pressure difference > 10 mmHg, bruit over subclavian arteries or aorta, and arteriographic abnormality. A patient was diagnosed with TA if at least three of these six criteria were present. We collected the patients' cases from the medical records from January 2003 to August 2015 at Nagasaki University Hospital. The data at admission or the first visit were used in this study. A total of 34 patients were registered in this study as of August 2015.

Clinical characteristics

The following characteristics of the 34 patients were examined: age, gender, family history, smoking, and angiographic classification that was determined according to the International TA Conference in Tokyo 1994 classification (Hata et al. 1996).

We also examined symptoms including fever, general malaise, headache, dizziness, neck pain, arm claudication and arthralgia, and signs including systolic blood pressure difference, bruits, pulse deficit and AR. The following laboratory data were also examined: white blood cell (WBC) count, hemoglobin, platelets, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), tortuosity of the aorta on chest X-ray, and macaroni sign on cervical ultrasound examination.

Activities of TA were evaluated by Kerr's criteria (Kerr et al. 1994). These criteria are used to define "active disease" when two of the following criteria are positive: (1) systemic features with no other cause, (2) elevated ESR, (3) indications of vascular ischemia or inflammation (e.g., claudication, diminished or absent pulses, bruit, vascular pain, asymmetric blood pressure), or (4) typical angiographic features (including any imaging method in addition to conventional angiography).

The treatments administered to the patients and outcome (relapse and death) were also examined. Japan College of Rheumatology-certified rheumatologists determined relapse by confirmation of the recurrence of active disease after remission (e.g., new vascular lesions, new bruit or new asymmetry in pulses, fever with an absence of infection).

HLA typing

We performed HLA-I allele typing for the 25 TA patients by conducting a reverse sequence-specific oligonucleotide with polymerase chain reaction (PCR-rSSO). LABType®SSO Typing Tests (One Lambda, Canoga Park, CA, USA) were used for the HLA typing. The protocol consisted of the DNA amplification process, hybridization, reading on a special device (LABScan™100), and interpretation by software (HLA Fusion™). All procedures were performed according to the manufacturers' instructions. Briefly, the target DNA was amplified by PCR using a group-specific biotinylated primer. The PCR product was subsequently denatured and allowed to rehybridize to complementary DNA probes conjugated to fluorescently coded microspheres.

The DNA-coated microspheres were detected in a Luminex analyzer, and the pattern of positive microspheres was used to determine the HLA typing. HLA-B52-allele-positivity was defined as the state of having at least one HLA-B52 allele using this method. The associations between these characteristics and the HLA-B52 allele were evaluated. Approval was obtained from the Ethics Committee of the institute in accord with the provisions of the World Medical Association's Declaration of Helsinki. All of the patients gave their informed consent to be subjected to the protocol, which was approved by the Institutional Review Board of Nagasaki University.

Statistical analysis

The data of the clinical features are expressed as the number of patients (%) with the indicated characteristic. Mann-Whitney's U-test was used to compare the patients' ages, the duration from onset to diagnosis, and the laboratory data. The chi-square test was used for categorical variables. P-values < 0.05 in these tests were considered significant. All statistical analyses were performed using JMP Pro, version 11 (SAS Institute, Tokyo).

Results

Demographic features

The average age of the 34 TA patients was 44.1 ± 18.3 years (Table 1); 31 patients (91.2%) were female. The average age of disease onset was 34.0 ± 17.6 years, and 24 patients (70.6%) were younger than 40 years at onset. One patient had a family history of TA. Eight patients (23.5%) were smokers. Angiographic classification showed that type V TA was the most common, followed by types I and II.

Clinical features

Among the symptoms, fever was the most common, followed by general malaise and headache. A systolic

Table 1. Clinical characteristics of 34 patients with Takayasu arteritis.

Male:Female (n)	3:31
Age at entry (y)	44.1 ± 18.3
Age at onset (y)	34.0 ± 17.6
Age at disease onset < 40 years	24 (70.6%)
Family history of TA	1 (2.9%)
Smoking	8 (23.5%)
Classification	
Type I	11 (32.3%)
Type II	7 (20.6%)
Type III	0 (0.0%)
Type IV	0 (0.0%)
Type V	16 (47.1%)
Symptoms	
Fever	20 (58.8%)
General malaise	19 (55.9%)
Headache	15 (44.1%)
Dizziness	11 (32.3%)
Neck pain	8 (23.5%)
Arm claudication	11 (32.3%)
Arthralgia	8 (23.5%)
Physical signs	
Systolic blood pressure difference > 10 mm Hg between arms	17 (50.0%)
Bruit over subclavian artery	20 (58.8%)
Bruit over abdominal aorta	11 (32.3%)
Pulse deficit	6 (17.6%)
Aortic valve regurgitation	11 (32.3%)

Values are means ± SD or number (percentage) unless indicated otherwise.

blood pressure difference between arms was detected in 50.0% of the patients. Bruit over the subclavian arteries was audible in 58.8% of patients.

Laboratory data

Initial laboratory data showed the following: WBC count 10,347 ± 6,659/μL, and CRP 6.4 ± 6.8 mg/dL (Table 2). Twenty-nine patients (85.3%) had been treated with glucocorticoids including steroid pulse therapy (9 patients, 26.5%), whereas immunosuppressive agents were prescribed together with glucocorticoids in 17 patients (50.0%) and biologics in 6 patients (17.6%). Surgical treatment was performed in 7 patients (20.6%). Thirty patients (88.2%) had active disease according to Kerr's criteria. Though relapse occurred in 10 patients (29.4%), all patients survived.

Table 2. Laboratory data and treatments of 34 TA patients.

Laboratory data	
WBC (/mm^3)	10,347 ± 6,659
Hemoglobin (g/dL)	11.4 ± 1.8
Platelet (× 10^3/mm^3)	316.1 ± 133.1
ESR (mm/hour)	61.9 ± 35.2
CRP (mg/dL)	6.4 ± 6.8 (0.02～22.61)
Positive HLA-B52 allele (n = 25)	17 (68.0%)
Tortuosity of aorta on chest X-ray	19 (55.97.1%)
Macaroni sign on ultrasound images of carotid arteries (n = 14)	7 (50.0%)
Treatment	
Corticosteroids (n)	29 (85.3%)
Corticosteroids (dose) (mg/day)	31.0 ± 13.0
Steroid pulse therapy	9 (26.5%)
Immunosuppressants	17 (50.0%)
Biologics	6 (17.6%)
Surgical treatment	7 (20.6%)
Active disease defined by Kerr's criteria	30 (88.2%)
Relapse	10 (29.4%)
Survival	34 (100.0%)

Values are means ± SD or number (percentage) unless indicated otherwise.

HLA typing and comparison with clinical characteristics

HLA typing was examined in 25 TA patients. The HLA-B52 allele was found in 17 of the 25 patients (68.0%). No significant differences in age at disease onset or subtype classification were found between the 17 HLA-B52-allele-positive patients and the eight HLA-B52-allele-negative patients (Table 3). The number of smokers was significantly higher among the HLA-B52-allele-positive patients (35.3%) than the HLA-B52-allele-negative patients (0.0%). All of the smokers were HLA-B52-allele-positive; there were no smokers among the HLA-B52-allele-negative patients. Among the three male TA patients, the single smoker was HLA-B52-allele-positive.

The frequencies of headache and dizziness were higher in the HLA-B52-allele-positive patients compared to the HLA-B52-allele-negative patients. The initial CRP level in the HLA-B52-allele-positive patients (9.0 ± 6.4 mg/dL) was significantly higher than that in the HLA-B52-allele-negative patients (3.2 ± 3.9 mg/dL) (Table 4).

All 17 patients in the HLA-B52-allele-positive group had active disease as defined by Kerr's criteria. The HLA-B52-allele-positive group had significantly higher initial prednisolone dosages (37.7 ± 8.6 mg/day) compared to the HLA-B52-allele-negative patients (23.1 ± 13.1 mg/day). Biologics were used only in HLA-B52-allele-positive patients.

Table 3. Association of HLA-B52 allele with clinical features in 25 TA patients.

	HLA-B52(+) (n = 17)	HLA-B52(−) (n = 8)	*p* value	Odds ratio	95% CI
Male:Female (n)	2:15	0:8	0.20	1.13#	0.95-1.35
Age at entry (y)	41.5 ± 19.1	40.5 ± 19.0	0.98	—	—
Age at onset (y)	35.4 ± 18.5	35.5 ± 18.2	0.93	—	—
Onset under 40 years	9 (52.9%)	5 (62.5%)	0.47	0.53	0.10-3.00
Duration from onset to diagnosis (y)	6.1 ± 11.2	5.1 ± 7.7	0.98	—	—
Family history	1 (5.9%)	0 (0.0%)	0.37	1.06#	0.94-1.20
Smoking	6 (35.3%)	0 (0.0%)	0.019*	1.55#	1.09-2.20
Classification					
Type I	5 (29.4%)	2 (25.0%)	0.82	1.25	0.19-8.44
Type II	5 (29.4%)	1 (12.5%)	0.34	2.92	0.28-30.30
Type III	0 (0.0%)	0 (0.0%)	—	—	—
Type IV	0 (0.0%)	0 (0.0%)	—	—	—
Type V	7 (41.2%)	5 (62.5%)	0.32	0.42	0.07-2.36
Symptoms					
Fever	12 (70.6%)	3 (37.5%)	0.12	4.00	0.68-23.51
General malaise	9 (52.9%)	5 (62.5%)	0.65	0.68	0.12-3.77
Headache	11 (64.7%)	1 (12.5%)	0.011*	12.83	1.26-130.51
Dizziness	6 (35.3%)	0 (0.0%)	0.019*	1.55#	1.08-2.20
Visual impairment	3 (17.7%)	0 (0.0%)	0.11	1.21#	0.97-1.51
Neck pain	4 (23.5%)	0 (0.0%)	0.064	1.31#	1.00-1.70
Arm claudication	5 (29.4%)	2 (25.0%)	0.34	2.92	0.28-30.30
Arthralgia	4 (23.5%)	2 (25.0%)	0.94	0.92	0.13-6.51
Physical signs					
Systolic blood pressure difference > 10 mm Hg between arms	12 (70.6%)	1 (12.5%)	0.041	9.60	0.85-108.72
Bruit over subclavian arteries	11 (64.7%)	3 (37.5%)	0.20	3.06	0.53-17.46
Bruit over abdominal aorta	5 (29.4%)	2 (25.0%)	0.82	1.25	0.19-8.44
Pulse deficit	3 (17.7%)	1 (12.5%)	0.74	1.50	0.13-17.18
Aortic valve regurgitation	9 (52.9%)	1 (12.5%)	0.043	7.88	0.79-78.67

Values are means ± SD or number (percentage) unless indicated otherwise.
*p < 0.05.
Relative risk is shown instead of odds ratio.

Discussion

The results of the present study revealed that the frequency of HLA-B52 allele in TA patients in Nagasaki Prefecture (68.0%) was the highest among all Japanese TA patients examined to date. It was reported that the frequency of the HLA-B52 allele was significantly higher in TA patients (28.6%) than in controls (10.7%) in the Japanese population (Takamura et al. 2012). Another study reported that the frequency of HLA-B52 allele in TA patients (56%) was significantly increased in comparison with healthy controls (25%) in the Japanese population (Kitamura et al. 1998).

We also investigated whether the presence of HLA-B52 allele was associated with the characteristics of TA. The CRP level in our HLA-B52-allele-positive group was higher than that in the HLA-B52-allele-negative group. All 17 patients in the HLA-B52-allele-positive group had active disease as defined by Kerr's criteria, and higher doses of corticosteroids were required for the HLA-B52-allele-positive group. In addition, although the differences were not significant, the frequencies of bruits and the systolic

Table 4. Association of HLA-B52 allele with laboratory data and treatments in 25 TA patients.

	HLA-B52(+) (n = 17)	HLA-B52(−) (n = 8)	*p* value	Odds ratio	95% CI
Laboratory data					
WBC (/mm^3)	9,988 ± 3,169	8,813 ± 2,195	0.52	−	−
Hemoglobin (g/dL)	11.2 ± 1.9	11.8 ± 1.8	0.48	−	−
Platelet(× 10^3/mm^3)	355.3 ± 137.1	300.6 ± 60.9	0.48	−	−
ESR (mm/hour)	74.3 ± 30.8	45.4 ± 43.0	0.13	−	−
CRP (mg/dL)	9.0 ± 6.4	3.2 ± 3.9	0.027*	−	−
Fibrinogen (mg/dL)	545.4 ± 122.6	455.0 ± 140.5	0.15	−	−
Macaroni sign on ultrasound images of carotid arteries (n = 12)	4/9 (44.4%)	1/3 (33.3%)	0.73	1.60	0.10-24.70
Active disease defined by Kerr's criteria	17 (100.0%)	6 (75.0%)	0.026	1.33#	0.89-2.00
Treatments					
Corticosteroids (n)	15 (87.5%)	7 (87.5%)	0.96	1.07	0.08-13.90
Corticosteroids (dose) (mg/day)	37.7 ± 8.6	23.1 ± 13.1	0.0067*	−	−
Steroid pulse therapy	8 (47.1%)	1 (12.5%)	0.077	6.22	0.62-62.16
Immunosuppressants	11 (64.7%)	3 (37.5%)	0.20	3.06	0.53-17.46
Biologics	5 (29.4%)	0 (0.0%)	0.036*	1.42	1.04-1.93
Surgical treatment	5 (29.4%)	1 (12.5%)	0.34	2.92	0.28-20.90
Relapse	7 (41.2%)	3 (37.5%)	0.42	2.10	0.32-13.61
Survival	17 (100.0%)	8 (100.0%)	−	−	−

Values are means ± SD or number (percentage) unless indicated otherwise.
*$p < 0.05$.
Relative risk is shown instead of odds ratio.

blood pressure differences in the HLA-B52-allele-positive group were higher than those in the HLA-B52-allele-negative group.

Many cases in the HLA-B52-allele-positive group followed the typical clinical course of TA. In prior studies, associations of HLA-B52 allele with more extensive aortic disease and refractoriness to corticosteroids were also observed (Numano et al. 1982; Moriwaki and Numano 1992; Kitamura et al. 1998), which might suggest an association of HLA-B52 allele with more active and severe disease.

Our study also revealed for the first time that the frequency of smokers among HLA-B52-allele-positive patients was significantly higher than among HLA-B52-allele-negative patients. Kumral et al. (2002) investigated the frequencies of the atherosclerotic vascular risk factors in 18 TA patients, and they reported that among them, smoking was present in 6 patients (33%), and hypertension, diabetes mellitus, and hypercholesterolemia were each observed in one patient (6%) respectively.

With regard to the pathogenesis of autoimmune diseases, the complex of genetic factors and environmental factors is important. Smoking is also likely to cause an inflammatory reaction with an increased release of reactive oxygen species, collagenases, serine proteases, and proinflammatory cytokines (Johannsen et al. 2014). Our present findings suggested that the presence of both HLA-B52 allele and smoking might play an important role in the development of TA.

This study involved a small number of patients and was limited by its retrospective nature, as the data that could be collected were dependent on the medical records. Larger numbers of cases are required in future studies in order to confirm the involvement of HLA-B52 allele in the activities of TA. Even though all of the variables were measured at the diagnosis of the disease, we were not sure if they accurately reflected the manifestations at the onset.

Most of the TA patients in our study were young females, and our analysis revealed that the HLA-B52 allele was frequent among TA patients in Nagasaki Prefecture, Japan. We also found for the first time that the frequency of smoking in the HLA-B52-allele-positive group was significantly increased compared to the HLA-B52-allele-negative group. The HLA-B52-allele-positive patients had more active disease and required more doses of corticosteroids.

Acknowledgments

We thank all the staff at Department of Immunology and Rheumaology, Graduate School of Biomedical Sciences, Nagasaki University, Japan. All of the authors contributed signifi-

cantly to the submitted work, and they have read and approved the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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