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Comparison of HLA-B*27 subtypes between Chinese patients with ankylosing spondylitis and non-ankylosing spondylitis carriers

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Abstract

Objective: To investigate the distribution of subtypes between HLA-B*27 (+) patients with ankylosing spondylitis (AS) and carriers.

Methods: This case–control study recruited Chinese Han patients with HLA-B*27 (+) AS from six hospitals in Zhejiang Province, China between 2013 and 2018. Patients who were examined for HLA-B*27 because of back pain or arthralgia but who did not have AS or arthritis were recruited as controls. HLA-B*27 target DNA was amplified by amplification refractory mutation systems and HLA-B*27 subtypes were determined by sequencing.

Results: The positive rate of HLA-B*27 was significantly higher in the AS group than in the control group. In AS patients, HLA-B*2704 was predominant at 86.4%, followed by HLA-B*2705 at 12.6%; HLA-B*2704 and HLA-B*2705 were found in 70.0% and 10.0% of controls, respectively. HLA-B*2702 and HLA-B*2706 were detected at low frequencies in the control group, while the rare subtype HLA-B*2715 was only observed in two (1.0%) patients with AS. HLA-B*2707 was not detected in AS or control groups.

Conclusion: HLA-B*2704 is the predominant subtype among patients with AS and carriers in southeast China.

Keywords

Ankylosing spondylitis, human leukocyte antigen, HLA-B*27, subtypes, case–control study, carriers

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Introduction

Ankylosing spondylitis (AS) is a debilitating and complex chronic autoimmune disease that affects the axial joints. Its prevalence is around 0.24% in the Chinese population,[1] and about 0.86% in Caucasians.[2] AS mainly occurs in young adults and leads to a high rate of disability, resulting in substantial personal and societal costs.

AS is thought to result from interactions among the environment, genes, sex, age, and ethnic background.[3] Sibling- and twin-based studies showed that AS susceptibility is clearly attributable to genetic factors,[2,4] and a strong association of the human leukocyte antigen (HLA)-B*27 with AS was revealed in 1973.[5] HLA-B*27 is highly polymorphic and more than 155 subtypes have been identified to date.[6] However, HLA-B*27 may not carry the same degree of susceptibility among different races and ethnic populations, perhaps as a result of varying genetic interactions and geographic origins.[7–14]

Indeed, HLA-B*2704 is the predominant subtype in the Chinese population,[15] while HLA-B*2715 is found exclusively in patients with AS.[13,15,16] Considering that the Chinese population is known for its genetic diversity, we chose to determine HLA-B*27 allele frequencies in AS patients in Zhejiang Province, southeast China. We predicted that they may exhibit variations from previous studies in other parts of China.[17–20] The capital of Zhejiang Province is Hangzhou, which is the fourth largest city in China. As an economic hub, Hangzhou is characterized by a large proportion of inhabitants from various parts of China, including many of the Han ethnic group, although the exact genetic diversity of Zhejiang Province remains to be determined.

Herein, we investigated the distribution of subtypes between HLA-B*27 (+) patients with AS and carriers, and compared differences in clinical features using genetic subtyping.

Materials and methods

Patients and control subjects

This case–control study was approved by the ethics review board of the Affiliated Zhuji Hospital with Wenzhou Medical University. Written informed consent was provided by each enrolled patient. The study was performed in accordance with the Declaration of Helsinki and its recommendations.

Chinese Han patients with HLA-B*27 (+) AS were enrolled from six rheumatology outpatient clinics in Zhejiang Province (Affiliated Zhuji Hospital with Wenzhou Medical University, Zhejiang Lishui Central Hospital, Lishui Hospital of Zhejiang University, Shaoxing People's Hospital, Shaoxing Hospital of Zhejiang University, and Hangzhou First People's Hospital) between January 2013 and June 2018. All patients were diagnosed according to the modified New York criteria for AS.[21]

Medical charts were reviewed and data regarding demography, the Bath ankylosing spondylitis disease activity index (BASDAI), the modified stoke ankylosing Spondylitis Spine Score (mSASSS, as scored by independent reviewers), lower back pain, uveitis, peripheral arthritis, and laboratory indicators including erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were collected. All data were obtained at diagnosis.

Patients who were examined for HLA-B*27 because they presented with back pain or arthralgia but who were determined to be otherwise healthy, without AS or other forms of arthritis, were recruited as controls. Control inclusion criteria were: 1) back pain or arthralgia; 2) negative results for rheumatoid factors; and 3) normal levels of ESR and CRP. Control exclusion criteria were: 1) morning stiffness

or lower back pain; 2) obvious sacroiliitis by imaging examination; and 3) relatives of HLA-B*27 (+) AS patients.

HLA-B*27 allele typing

Genomic DNA was extracted from whole blood using a commercial DNA extraction kit (Tiangen Biotech Co., Ltd., Beijing, China). HLA-B*27-specific primers were designed based on the IMGT/HLA sequence database, version 3.13.1 (ftp://ftp.ebi.ac.uk/pub/databases/ipd/imgt/hla/). Analysis by ClustalX software showed the 454 G site (NC_000006.12) of HLA-B*27 to be the most specific. The amplification refractory mutation system was used to amplify target DNA using the primers HLA-B*27-F1 (5′-GG AGTAT TGGGACCGGGAGACACA GATCAG-3′) and HLA-B*27-RO5 (5′-ATCT ACAG AGCCAC TCCA CG C-3′).[22] PCR conditions were: pre-denaturation at 94°C for 5 minutes, then 35 cycles of 94°C for 1 minute, 60°C for 1 minute, and 72°C for 1 minute, followed by annealing/extension at 72°C for 10 minutes. HLA-B*27 subtyping was achieved by sequencing the resulting PCR products (Beijing Genomics Institute, Beijing, China) using the sequencing primers HLA-B*27-seqF (5′-GGAGT ATTGGGACCGGGAGA-3′), HLA-B*27-RO5 (5′-ATCTACAGAGCCACTCCACG C-3′), or HLA-B*27-FO (5′-CTCCCACTC CATGAGGTATTTCC-3′) and HLA-B*27-R1 (5′-CCTCTCGGTCAGTCTGTGCCTT GGCCTTCC-3′).

Statistical analysis

SPSS software version 19.0 (IBM Corp., Armonk, NY, USA) was used for all statistical analyses. Continuous variables with normal distribution were expressed as means ± standard deviation (SD) and were analyzed using the Student's t test. Continuous variables with non-normal distribution were expressed as medians (interquartile range [IQR]) and were analyzed using the Mann–Whitney U test. Categorical variables were expressed as frequency (percentage) and were analyzed using the chi-squared test or Fisher's exact test, as appropriate. $P<0.05$ was considered statistically significant.

Results

A total of 235 patients with AS and 261 controls were included in the study. Baseline characteristics of the subjects are shown in Table 1. There were no significant differences between the two groups in terms of age and sex.

The positive rate of HLA-B*27 was significantly higher in the AS group than in the control group ($P<0.001$). Five HLA-B*27 subtypes were identified in AS and control groups. In AS patients, HLA-B*2704 was predominant, occurring in 86.4% of cases, followed by HLA-B*2705 at 12.6%; HLA-B*2704 and HLA-B*2705 were found in 70.0% and 10.0% of controls, respectively. HLA-B*2702 and HLA-B*2706 were detected at low frequencies in the control group, while the rare subtype HLA-B*2715 was observed in only two (1.0%) patients with AS. HLA-B*2707 was not detected in AS or control groups (Table 2). The frequencies of the main subtypes (HLA-B*2704 and HLA-B*2705) were similar between AS patients and controls. The reported low P value was a result of outliers with rare HLA-B*27 subtypes.

Clinical features and laboratory parameters of HLA-B*2704 and HLA-B*2705 subtypes of AS patients are shown in Table 3. There were no significant differences between the two subtypes.

Regarding the clinical presentation, lower back pain was significantly more common in AS patients with HLA-B*2704 and HLA-B*2705 subtypes compared with the HLA-B*27-negative group ($P<0.05$). Uveitis was significantly less common in

Table 1. Characteristics of study subjects.

Characteristic	AS (n = 235)	Control (n = 261)	*P*
Age (years), median (IQR)	38.9 (29.0, 46.0)	36.0 (29.0, 45.0)	0.573[a]
Age at onset (years), median (IQR)	31.3 (13.0, 38.0)		
Sex, n (%)			0.558[b]
Female	97 (41.3)	101 (38.7)	
Male	138 (58.7)	160 (61.3)	
Lower back pain, n (%)	154 (65.5)		
Uveitis, n (%)	20 (8.5)		
Peripheral arthritis, n (%)	61 (25.9)		
ESR (mm/h), median (IQR)	38.0 (32.0, 51.0)		
CRP (mg/L), median (IQR)	22.0 (12.0, 31.0)		
BASDAI, median (IQR)	4.7 (3.5, 7.2)		
mSASSS, median (IQR)	30.5 (22.8, 37.5)		

[a]Mann–Whitney U test.
[b]Chi-squared test.
AS: ankylosing spondylitis; BASDAI: Bath disease activity index; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; IQR: interquartile range; mSASSS: modified stoke ankylosing spondylitis spine score; SD: standard deviation.

Table 2. Distribution of HLA-B*27 subtypes in the southeast China population.

Subtype, n (%)	AS (n = 235)	Control (n = 261)	*P*
HLA-B*27			<0.001[a]
+	207 (88.3)	10 (3.8)	
–	28 (11.7)	251 (96.2)	
HLA-B*27 (+)[#]			<0.001[a]
HLA-B*2702	0	1 (10.0)	
HLA-B*2704	179 (86.4)	7 (70.0)	
HLA-B*2705	26 (12.6)	1 (10.0)	
HLA-B*2706	0	1 (10.0)	
HLA-B*2707	0	0	
HLA-B*2715	2 (1.0)	0	

[#]There were 207 individuals in the AS group and 10 in the control group.
[a]Chi-squared test.
AS: ankylosing spondylitis; HLA: human leukocyte antigen.

HLA-B*2704- and HLAB*2705-positive patients with AS than in HLA-B*27-negative patients with AS ($P < 0.05$) (Table 4).

Discussion

This study aimed to investigate the distribution of subtypes between HLA-B*27 (+) patients with AS and carriers. The results suggest that HLA-B*2704 and HLA-B*2705 subtypes are associated with AS in southeast China, but there were no significant differences between HLA-B*2704 and HLA-B*2705 haplotypes in patients with AS.

HLA-B*27 has a high degree of polymorphism,[22] and its subtypes are associated with various racial and geographical

Table 3. Comparison of clinical and laboratory parameters between HLA-B*2704- and HLA-B*2705-positive patients with AS.

Variable	HLA-B*2704 (n = 179)	HLA-B*2705 (n = 26)	*P*
Age (years), median (IQR)	34.0 (22.0, 45.0)	37.0 (34.5, 47.3)	0.121[a]
Age at onset (years), median (IQR)	24.0 (19, 36.0)	22 (15.0, 38.3)	0.380[a]
Sex, n (%)			0.763[b]
Female	77 (43.0)	12 (46.2)	
Male	102 (57.0)	14 (53.8)	
ESR (mm/h), median (IQR)	38.0 (32.0, 52.0)	35.0 (31.0, 45.3)	0.617[a]
CRP (mg/L), median (IQR)	21.0 (11.0, 31.0)	21.0 (9.8, 25.3)	0.713[a]
BASDAI, median (IQR)	6.0 (4.0, 7.0)	6.0 (4.8, 7.0)	0.532[a]
mSASSS, median (IQR)	31.5 (23.3, 36.8)	32.5 (25.0, 42.5)	0.538[a]

[a]Mann–Whitney U test.
[b]Chi-squared test.
AS: ankylosing spondylitis; BASDAI: Bath disease activity index; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; HLA: human leukocyte antigen; IQR: interquartile range; mSASSS: modified stoke ankylosing spondylitis spine score; SD: standard deviation.

Table 4. Association of the clinical presentation with HLA-B*27 subtypes in patients with AS.

Subtype, n (%)	HLA-B*2704 (n = 179)	HLA-B*2705 (n = 26)	HLA-B*2715 (n = 2)	HLA-B*27 (−) (n = 28)	*P*
Lower back pain	129 (72.1)*	18 (69.2)*	1 (50.0)	6 (21.4)	<0.001a
Uveitis	2 (1.1)*	2 (7.7)*	0	16 (57.2)	<0.001[a]
Peripheral arthritis	48 (26.8)	6 (23.1)	1 (50.0)	6 (21.4)	0.782[a]

[a]Chi-squared test.
*$P<0.05$ vs. HLA-B*27 (–) group.
HLA: human leukocyte antigen.

features. Moreover, the association of certain alleles with AS has been well established in many ethnic groups.[13,23] In the present study, we compared HLA-B*27 subtype frequencies in Chinese Han AS patients and non-AS controls from different regions of southeast China. Because the frequency of HLA-B*27 is 5% in healthy individuals,[3,24–26] we selected non-AS patients with back pain rather than completely healthy subjects. We found that HLA-B*2704 was the predominant subtype, followed by HLA-B*2705 in both AS patients and non-AS controls where similar frequencies were observed. This result differs from that reported in a Han Chinese study from Lanzhou,[20] northern China. However, notably, the overall frequency of HLA-B*2704 in patients with AS was 86.4%, which is in agreement with other studies of Asian populations,[14,16,18,27,28] except populations in Thailand and Indonesia.[29,30]

There were no significant differences between HLA-B*2704 and HLA-B*2705 haplotype frequencies in patients with AS regarding disease markers. However, the frequency of lower back pain at presentation was higher in HLA-B*2704 and HLA-B*2705 patients than in HLA-B*27-negative

patients. Previous studies showed that the HLA-B*2704 haplotype has a higher penetrance[31,32] Nevertheless, Monteserrat et al.[33] reported that basic immunologic features were similar between patients with HLA-B*2704 and HLA-B*2705 subtypes. Globally, these previous studies support the results of the present study.

We did not detect HLA-B*2702, HLA-B*2707, or HLA-B*2706 subtypes in AS patients. Previous studies on the role of HLA-B*2706 in AS are controversial,[29,34–37] which might reflect the fact that this subtype is relatively rare and the number of patients and controls with HLA-B*2706 is limited.

Notably, we detected the rare HLA-B*2715 subtype in two male AS patients in the Lishui region of Zhejiang Province, but not in the other three regions. HLA-B*2715 was first reported by Garcia-Fernandez et al.[16] It was found to have a positive rate of 2.24% in Thai AS patients,[29] and was shown by Mou et al. to be associated with juvenile and adult AS in southern China.[15] Wu et al. also identified HLA-B*2715 in a multiplex Han family,[38] while Yi et al.[20] reported this polymorphism in Chinese Han patients with AS. Gonzalez-Roces et al. verified that each allele was considered to be a susceptible or predisposing subtype if detected in one patient of a particular population.[27] Hence, we consider HLA-B*2715 to be a disease-associated subtype, at least in southeastern Han Chinese.

Lishui is a mountainous region with a complex terrain whose geographic and environmental variance creates genetic diversity. This provides an intriguing insight into the connection between heredity and disease. Therefore, we suggest that HLA-B*2715 plays an important role in the pathogenesis of AS in accordance with specific geographic features. Further investigation should be conducted by recruiting AS patients from certain areas to elucidate the definitive association between HLA-B*2715 and AS.

Polymorphisms in HLA-B*27 subtypes influence the biochemical, biophysical, and antigenic properties of the molecule, leading to different levels of penetrance in the development of HLA-B*27-related diseases.[39–41] Because different polymorphisms have varying involvement in disease development, this could have implications for future targeted treatments of AS.

In conclusion, HLA-B*2704 is the predominant subtype among patients with AS and non-AS controls in southeast China.

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Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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