RESEARCH ARTICLE

Polymorphisms of human leukocyte antigen B*27 on clinical phenotype of spondyloarthritis in Chinese

Hai-Jun Ma^{1,2} | Qing-Feng Yin³ | Yun Liu² | Yin Wu⁴ | Tie-Chui Zhu² | Ming-Hao Guo²

¹Department of Rheumatology & Immunology, Medical School of Nanjing University, Nanjing, China

²Division of Rheumatology, the First Affiliated Hospital, Xixiang Medical University, Weihui, China

³Division of Endocrinology, the First Affiliated Hospital, Xixiang Medical University, Weihui, China

⁴Division of Rheumatology, the Third Affiliated Hospital, Xixiang Medical University, Xixiang, China

Correspondence

Hai-Jun Ma, Department of Rheumatology & Immunology, Medical School of Nanjing University, Nanjing, China.

Email: haijunma97@hotmail.com and

Ming-Hao Guo, Division of Rheumatology, the First Affiliated Hospital, Xixiang Medical University, Weihui, China.

Email: guominghao11@yahoo.com.cn

Background: In recent years, an ever-increasing number of alleles of human leukocyte antigen B*27 (HLA-B*27) have been identified. This study aimed to establish an updated method for HLA-B*27 subtyping, and to investigate the impact of HLA-B*27 polymorphisms on the clinical phenotype of spondyloarthritis (SpA).

Methods: Overall, 184 SpA patients were recruited for analyzing diversity of HLA-B*27 via an updated high-resolution polymerase chain reaction amplification with sequence specific primers (PCR-SSP).

Results: The prevalence of HLA-B*27 was 94.0%, and four subtypes were identified including HLA-B*2704 (77.5%), B*2705 (20.2%), B*2707 (1.7%), and B*2724 (0.6%). There was an obvious male predominance ($P=.05$) and markedly elevated C-reactive protein (CRP) in B*27 positive SpA ($P<.01$). In multivariate linear regression analysis, the elevated CRP was positively associated with HLA-B*27 positivity (regression coefficient $B=46.1$, $P=.0003$), grade of sacroiliitis ($B=47.5$, $P=.0032$), and male gender ($B=20.4$, $P=.0041$). Notably, a male predilection was also found in B*2705 positive SpA while B*2707 was associated with older age, higher positive family history, and higher prevalence of extra-articular features (all $P<.05$).

Conclusions: In this study, an updated PCR-SSP technique to identify increasing alleles of HLA-B*27 was developed and their different effects on clinical manifestations of SpA were demonstrated. Genotyping of HLA-B*27 would shed light on our understanding of the pathogenesis of SpA.

KEYWORDS

HLA-B*27, sequence specific primer, spondyloarthritis, subtype

1 | INTRODUCTION

Spondyloarthritis (SpA) is a group of common chronic inflammatory rheumatic diseases, including ankylosing spondylitis (AS), psoriatic spondylitis (PsA), reactive arthritis, arthritis related to inflammatory bowel disease, and undifferentiated spondyloarthritis (uSpA). They are characterized mainly by affected axial skeleton, male predominance, and high tendency of familial aggregation.¹

Since its strong association with AS was reported in 1973,² the prevalence of human leukocyte antigen B*27 (HLA-B*27) and its role

in the pathogenesis of SpA were extensively investigated in worldwide populations.³ HLA-B*27 served as an important parameter for screening suspected SpA patients⁴ and was also a predictor of poor prognosis.⁵ Furthermore, in European descent, HLA-B*27 detection was recommended as the first and sole test for those suspected axial SpA.⁶ Of particular note, as the clinical arm, HLA-B*27 has been included in the new classification criteria for axial and peripheral SpA.⁷ Aside from its assistance in clinical diagnosis, the role of HLA-B*27 in the pathogenesis of SpA has also been verified. Previous reports indicated that approximately 30% of the genetic susceptibility of SpA was ascribed

to HLA-B*27,^{8,9} and the development of spondyloarthropathy-like disease in HLA-B*27 transgenic rats required high copies of B*27.¹⁰ Additionally, in a prior study, greater expression of HLA-B*27 was found in AS patients.¹¹ Furthermore, recent studies showed that non-B*27 susceptible genes might participate in the pathogenesis of SpA through interaction with HLA-B*27 or in an HLA-B*27-dependent manner.¹²⁻¹⁴ These data, together with the finding that the subtypes of HLA-B*27 would influence the onset age of AS,¹⁵ suggested that HLA-B*27 might not merely be a genetic marker but rather a related primary pathogenic factor.

Nowadays, it has been recognized that HLA-B*27 has numerous alleles. Of more importance, subtypes of HLA-B*27 showed varied distribution in different races and regions, and which also exhibited differences in genetic susceptibility to SpA.¹⁶ A recent study described a set of primers to detect 42 HLA-B*27 alleles (B*2701-B*2721 and B*2723-B*2743).¹⁷ However, with continued advances in genotyping, more and more HLA-B*27 subtypes have been identified.¹⁸ In this context, subtyping of HLA-B*27 is need to be regularly updated to meet the requirements for investigating HLA-B*27 polymorphisms in the field of SpA.

Therefore, the present study aimed to establish an updated high-resolution polymerase chain reaction amplification with sequence specific primers (PCR-SSP) for HLA-B*27, to investigate the impact of HLA-B*27 status on the clinical presentation of SpA in Han Chinese, and to further analyze the polymorphisms of HLA-B*27 in governing phenotypic variations.

2 | MATERIALS AND METHODS

2.1 | Patients

Overall, 184 consecutive patients with SpA attending or followed by our department from January 2010 to January 2014 were enrolled in the present cross-sectional study. All of them belonged to Han nationality. All AS patients met the modified New York criteria,¹⁹ and who were classified as juvenile ankylosing spondylitis (JAS) and adult ankylosing spondylitis (AAS) based on their onset age under or over 16 years. Patients including undifferentiated spondyloarthritis (uSpA) and psoriatic spondylitis (PsA) were classified according to the European Spondyloarthropathy Study Group (ESSG) criteria for SpA.²⁰ The protocol was approved by the local ethics committee. All patients gave their written informed consents prior to enrollment in this study and their anonymity was preserved.

2.2 | Clinical data

Patients were interviewed via a standard questionnaire including demographic information and medical histories. Clinical features including the initial symptoms, age at onset, course of disease, and family history of SpA were documented at presentation or by checking medical charts retrospectively. The corresponding definitions of the initial symptoms and family history of SpA are in line with the Assessment in SpondyloArthritis international Society (ASAS) handbook.²¹

Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were measured. Radiological sacroiliitis grading was undertaken randomly and blindly by two experienced rheumatologists according to the New York scoring method for sacroiliac joints.²² If there was a discrepancy, the two specialists discussed and gave a conclusive grade or score.

2.3 | Typing and subtyping of HLA-B*27

Fasting peripheral venous blood were collected from all patients, and genomic DNA was extracted from whole blood samples using a standard salting-out method. On the basis of previously published studies,^{17,23-25} sequence specific primers for HLA-B*27 subtyping were updated in order to distinguish 56 subtypes of HLA-B*27 (B*2701-B*2721 and B*2723-B*2757). The corresponding specificities of primer pairs are listed in Table 1. The primers amplifying a 796-bp fragment of HLA-DRB1 gene were applied to produce internal controls in the high-resolution HLA-B*27 subtyping. The universal negative and blank controls serving as quality controls were included in each amplification. The PCR product was analyzed by 2.5% agarose gel electrophoresis followed by ethidium bromide staining.

2.4 | Statistical analysis

Quantitative data were presented as means $\pm$ SD and qualitative data were described with ratios or percentages. Quantitative data were analyzed with two independent samples using *t* test if the assumptions of homogeneity of variances and normality are met, otherwise the Wilcoxon rank sum test is used. With regard to qualitative data, the chi-square test, the continuity-adjusted chi-square, or Fisher's exact test when necessary was used. In order to control the effects of potential confounding variables, multivariate linear/logistic regression were conducted to analyze the independent effects of HLA-B*27 status or subtypes, gender, age at onset, course of disease, and family history of SpA on acute phase reactants, radiographic sacroiliitis, disease pattern, and extra-articular features. Odds ratios (OR) and regression coefficients (B) were calculated in logistic and linear regression models, respectively. A two-tailed *P* value <.05 was considered statistically significant. Statistical analyses were performed using SASv9.2.

3 | RESULTS

3.1 | HLA-B*27 typing and subtyping results

There were 162 AS (88.0%), 21 uSpA (11.4%), and 1 PsA patients (0.6%) included in this study. Among the 184 SpA patients, 11 patients (6.0%) were negative for HLA-B*27 and the remaining 173 B*27 positive patients (94.0%) were subsequently subtyped for HLA-B*27 alleles. Four subtypes of HLA-B*27 were discovered, including HLA-B*2704, B*2705, B*2707, and B*2724. The number of positive for HLA-B*2704, B*2705, B*2707, and B*2724 was 134 (77.5%), 35 (20.2%), 3 (1.7%), and 1 (0.6%), respectively.

TABLE 1 Sequence and specificity of primers and PCR product sizes for high-resolution subtyping of HLA-B*27

ID	Position	Sequence(5'→3')	B*27 alleles amplified	Product size(bp)
G1	149-167(E2, F) 272-292(E2, R)	GCTACGTGGACGACACGCT CAGTCTGTGCCTTGCGTTGC	01-11, 13-15, 17, 19-21, 24-28, 30-57, B*4497	144
G2	149-167(E2, F) 204-220(E2, R)	GCTACGTGGACGACACGCT GCGCCCGCGGCTCCTCT	01-51, 53-57, B*0773, B*3822, B*4093 B*44020101, B*4440	72
1	265-283(E2, F) 409-426(E2, R)	CAGATCTGCAAGGCCAAGG GTAGGCGTCCTGGTGGTA	01-05, 08-10, 13-15, 17, 19, 25, 28, 30 32, 36-40, 42, 44-57	407
2	149-167(E2, F) 301-317(E2, R)	GCTACGTGGACGACACGCT AGCAGGGTCCGCAGGTC	01-03, 05, 07, 09-10, 13-14, 16-17, 19, 27-29, 32 34-35, 37-39, 41-43, 45-56, B*4701/0101/02, B*4705	169
3	265-283(E2, F) 311-328(E2, R)	CAGATCTGCAAGGCCAAGG TG TAGTAGCGGAGCGCGA	02, 30, 53, 57	64
4	-3-14(E1, F) 272-292(E2, R)	GAGATGCGGGTCACGGA CAGTCTGTGCCTTGCGTTGC	13	423
5	254-272(E2, F) 409-428(E3, R)	ACCGGGAGACACAGATCTG TCGTAGGCGTACTGGTTATG	07, 11, 20, 24, 27, 33, 43, B*15243, B*1413? B*1509? B*3914?, B*5122? B*7803?	420
6	231-247(E2, F) 301-317(E2, R)	GCAGGAGGGGCCGAGC AGCAGGGTCCGCAGGTC	03	87
7	265-283(E2, F) 527-544(E3, R)	CAGATCTGCAAGGCCAAGG CTCTCAGCTGCTCCGCCT	04, 06, 10, 15, 20-21, 24, 25?, 40, 54	525
8	78-97(E2, F) 248-265(E2, R)	CCACTCCATGAGGTATTTCC GTGTCTCCCGTCCCAA	17	188
9	254-272(E2, F) 499-516(E3, R)	ACCGGGAGACACAGATCTG CTCCCACTTGCGCTGGGA	24, 36	508
10	274-292(E2, F) 362-381(E3, R)	AAGGCCAAGGCACAGACTT CACGTGCGAGCCATACATAT	01, 44	353
11	344-363(E3, F) 418-435(E3, R)	GGTCTCACACCCTCCAGAAT CTTGCCGTCGTAGGCGTG	09	92
12	265-283(E2, F) 353-372(E3, R)	CAGATCTGCAAGGCCAAGG GCCGTACATCCTCTGGATGA	19, 30, B*4497	353
13	265-283(E2, F) 559-576(E3, R)	CAGATCTGCAAGGCCAAGG GAGCCACTCCACGCACGT	15, 28	557
14	344-363(E3, F) 559-576(E3, R)	GGTCTCACACCCTCCAGAAT GAGCCACTCCACGCACAG	25, 38, 47, B*15129	233
15	265-283(E2, F) 369-385(E3, R)	CAGATCTGCAAGGCCAAGG GCCCCACGTGCGAGCCG	07, 11, 19-21, 24, 30, 32-34, 43	365
16	294-311(E2, F) 362-381(E3, R)	CCGAGAGAGCCTGCGGAA CACGTGCGAGCCATACATAT	08, 12, 18, 26, 40, 42, 44, B*15129, B*1802 B*4821, Cw*0705?	333
17	283-301(E2, F) 362-381(E3, R)	GCACAGACTGACCGAGAGG CACGTGCGAGCCATACATAT	03, 05, 09-10, 13, 16-17, 27-29, 35, 37-39 41-42, 45-56, B*3702?, B*4705?	344
18	254-272(E2, F) 418-435(E3, R)	ACCGGGAGACACAGATCTG CTTGCCGTCGTAGGCGTC	01-05, 08, 10, 12-17, 19, 25-26, 28, 30-32 36-40, 42, 44-45, 47-57, B*4497	427
19	346-363(E3, F) 419-436(E3, R)	GGTCTCACACCCTCCAGAAT CCTTGCCGTCGTAGGCGA	35, B*3822	93
20	78-97(E2, F) 272-292(E2, R)	CCACTCCATGAGGTATTTCT CAGTCTGTGCCTTGCGTTGC	37, B*4497	215

(Continues)

TABLE 1 (Continued)

ID	Position	Sequence(5'→3')	B*27 alleles amplified	Product size(bp)
21	149-167(E2, F)	GCTACGTGGACGACACGCT	39	155
	287-303(E2, R)	GTCCTCTCGGTCACTCA		
22	265-283(E2, F)	CAGATCTGCAAGGCCAAGG	08, 33, 40, 42, 44, B*7301	64
	311-328(E2, R)	TGTAGTAGCCGCGCAGGT		
23	254-272(E2, F)	ACCGGGAGACACAGATCTG	43	393
	385-401(E3, R)	AGGAGGCGCCCGTCCGA		
24	346-363(E3, F)	TCTCACACCCTCCAGAAT	25, 45, B*15129	210
	539-555(E3, R)	CTCCAGGTAGGCTCTCC		
25	149-167(E2, F)	GCTACGTGGACGACACGCT	48	95
	227-243(E2, R)	CGGCCCTCTCTGCTCTC		
26	346-363(E3, F)	GGTCTCACACCCTCCAGAAT	47	212
	538-555(E3, R)	CTCCAGGTAGGCTCTGTC		
27	254-272(E2, F)	ACCGGGAGACACAGATCTG	49	80
	316-333(E2, R)	CTGGTTGTAGTAGCGGAA		
28	254-272(E2, F)	ACCGGGAGACACAGATCTG	50, B*151804?	500
	489-508(E3, R)	TGCGCTGGGTGATCTGAGCT		
29	196-212(E2, F)	AGTCCGAGAGAGGAGCA	51	431
	362-381(E3, R)	CACGTCCGAGCCATACATAT		
30	372-391(E3, F)	CTGCGACGTGGGGCGGGACC	54	173
	527-544(E3, R)	CTCTCAGCTGCTCCGCCT		
31	254-272(E2, F)	ACCGGGAGACACAGATCTG	55	500
	488-508(E3, R)	TGCGCAGGGTGATCTGAGCCA		
32	344-363(E3, F)	GGTCTCACACCCTCCAGAAT	56	156
	482-499(E3, R)	TGATCTGAGCCGCCGTGC		

The first two pairs of primers (G1 and G2) are used to HLA-B*27 typing, and the remaining pairs of primers are used to HLA-B*27 subtyping. E2 means exon 2 and E3 means exon 3. F and R represent forward and reverse primers, respectively.

3.2 | Comparisons between B*27 positive and negative SpA patients

Both B*27 positive and negative SpA patients were quite young with respect to the mean age at the time of recruitment (28.6 ± 10.4 vs 27.5 ± 10.8 years, $P > .05$). As indicated in Table 2, there was an obvious male predominance in B*27 positive SpA patients as compared to B*27 negative counterparts ($P = .05$). As for acute phase reactants, the level of CRP was remarkably elevated in B*27 positive SpA patients when compared to B*27 negative SpA patients ($P < .01$). In multivariate linear regression model, the level of CRP was positively associated with HLA-B*27 positivity (regression coefficient $B = 46.1$, $P = .0003$), grade of sacroiliitis ($B = 47.5$, $P = .0032$), and male gender ($B = 20.4$, $P = .0041$). As shown in Table 2, the prevalence of upper back pain in B*27 negative SpA was significantly higher than that in B*27 positive patients among the initial spinal symptoms (18.2% vs 2.3% , $P < .05$). On the contrary, lower back pain was more common in B*27 positive (36.4%) than that in B*27 negative SpA patients (18.2%), but this deviation did not reach statistical significance ($P > .05$). Regarding the peripheral arthritis, the prevalence of hip pain in B*27 positive SpA patients was more common than that in B*27-negative patients at

disease onset (27.2% vs 0.0% , $P = .09$). Although the achilles tendonitis was a characteristic feature of SpA, there was no evidence of significant difference between both groups in terms of the initial enthesitis. No other significant differences were found between both groups as listed in Table 2.

3.3 | Comparisons between different HLA-B*27 subtypes

In consideration of the potential effects of HLA-B*27 subtypes on the disease expression of SpA, comparisons of clinical manifestations were established among HLA-B*2704, B*2705, and B*2707 positive SpA patients. As presented in Table 3, the mean age was older in B*2707 positive SpA than that in B*2704 and B*2705 counterparts (44.7 ± 6.0 vs 28.8 ± 10.1 and 26.8 ± 10.9 years, both $P < .05$). In addition, the male to female ratio was quite different between these three subtypes ($P = .01$). Obviously, there was a male predominance in B*2705 positive SpA patients. There were no significant variances observed in terms of age at onset, course of disease, diagnostic delay, ESR, and radiographic sacroiliitis. In multivariate logistic regression analysis, course of disease ($OR = 1.145$, $P < .0001$) and axial pattern at disease

TABLE 2 Comparison of the demographic and clinical characteristics in B*27 positive and negative SpA patients.

	B*27(+) n=173 (%)	B*27 (-) n=11 (%)
Age (yr)	28.6±10.4	27.5±10.8
Male to Female*	140:33	6:5
Age at onset (yr)	21.1±8.9	22.6±9.4
Course of disease	7.5±7.3	4.3±4.1
Diagnostic delay	6.6±6.8	4.4±4.3
ESR (mm/h)	65.4±53.3	34.4±24.2
CRP (mg/L) [§]	40.2±36.9	9.3±9.6
Radiographic sacroiliitis		
Grade 0	7 (4.1)	0
Grade I	12 (6.9)	0
Grade II	70 (40.5)	2 (18.2)
Grade III	61 (35.3)	6 (54.5)
Grade IV	23 (13.3)	3 (27.3)
Disease pattern		
Axial	74 (42.8)	5 (45.5)
Peripheral	91 (52.6)	5 (45.5)
Mixed	8 (4.6)	1 (9.1)
Family history of SpA	49 (28.3)	2 (18.2)
Initial symptoms		
Spinal column involvement	82 (47.4)	6 (54.5)
Upper back pain [§]	4 (2.3)	2 (18.2)
Lower back pain	63 (36.4)	2 (18.2)
Buttock pain	12 (6.9)	2 (18.2)
Peripheral arthritis	96 (55.5)	4 (36.4)
Hip	47 (27.2)	0
Shoulder	3 (1.7)	0
Heel pain	7 (4.0)	1 (9.1)
Other enthesitis	2 (1.2)	1 (9.1)
Extra-articular features	17 (9.8)	0/11
Fever	5 (2.9)	0
Uveitis	4 (2.3)	0
Renal disease	2 (1.2)	0
Diagnosis categories		
AS/uSpA	153/19	9/2
JAS/AAS	57/96	3/6

Quantitative data were presented as means±SD and qualitative data were described with ratios or percentages.

* $P=0.05$, and [§] $P<0.05$ vs B*27(+) SpA patients group.

onset (OR=1.937, $P=0.0344$) were positively associated with radiological sacroiliitic lesions. With respect to the positive family history of SpA, it was significantly higher in B*2707 positive patients than that in the other two groups (100.0% vs 29.1% and 20.0%, both $P<0.05$). Likewise, the same trend was also observed in B*2707 positive SpA patients as for the extra-articular features (66.7% vs 6.7% and 17.1%,

both $P<0.05$). Comparing the diagnosis categories of patients, the ratio of JAS/AAS was much greater in B*2705 positive patients than that in B*2704 and B*2707 positive cases ($P=0.06$). As shown in Table 3, there were no significant differences observed between HLA-B*27 alleles with regard to the initial symptoms.

4 | DISCUSSION

HLA-B*27 is one of HLA class I molecule, which was broadly studied in different populations throughout the world due to its strong association with SpA.³ It has been reported that HLA-B*27 was the most powerful parameter for distinguishing SpA from non-SpA patients in the ESSG classification criteria.²⁰ Moreover, it has also been suggested that the absence of HLA-B*27 is associated with a significant diagnostic delay.¹ Therefore, HLA-B*27 testing was routinely performed in a large number of clinical laboratories. Traditional methods for typing HLA-B*27 included complement-dependent cytotoxicity (CDC) assay and flow cytometry (FCM).²⁶ Both of which determined serological reactivity of B*27, in other words, they detected cell surface antigen expression of B*27. Previous studies demonstrated that cell surface expression of B*27 was considerably diminished during *Yersinia* or *Salmonella* infection.^{26,27} This finding combined with the inevitable cross reactivity of anti-HLA-B*27 antibodies compromised serological typing results of B*27 to some extent.²⁸

Another point that should be mentioned is that the number of HLA-B alleles is increasing continuously and therefore more and more subtypes of HLA-B*27 were found, and which showed different associations with SpA.¹⁶ To solve this situation, polymerase chain reaction (PCR) was introduced to promote the development of typing and subtyping of HLA-B*27. Up to the present, several methods have been developed on the basis of PCR,²⁹⁻³³ namely, PCR with restriction fragment length polymorphism (PCR-RFLP), PCR with sequence specific oligonucleotide probes (PCR-SSOP), PCR with single-strand conformation polymorphism (PCR-SSCP), PCR with sequence specific primers (PCR-SSP), and PCR with sequence based typing (PCR-SBT). In all, these PCR-based methods did not need fresh blood samples or viable lymphocytes, a prerequisite for CDC and FCM. Of note, PCR-SSP was the most widely used method to assign HLA-B*27 subtypes among DNA-based typing approaches,^{17,23-25,32,34} and which could be easily updated by designing appropriate primers for lately reported alleles. Meanwhile, PCR-SSP could be practically performed in most laboratories without needing expensive instruments and special reagents as compared with other molecular typing methods.

Our present study established and tested an updated high-resolution PCR-SSP typing set to accurately assign 56 alleles or subtypes of HLA-B*27 from B*2701 to B*2757 (B*2722 was deleted due to its sequence was identical to B*2706). Based on our results, the prevalence of HLA-B*27 was 94.0% in the total SpA patients, which was higher than that in ESPERANZA and emAR II studies, but similar to another investigation from China.³⁵⁻³⁷ Furthermore, four subtypes of HLA-B*27 were detected in the total 184 SpA patients, namely,

TABLE 3 Comparison of the demographic and clinical characteristics in B*27 positive SpA patients, according to HLA-B*27 subtypes

	B*2704 n=134 (%)	B*2705 n=35 (%)	B*2707 n=3 (%)	B*2724 n=1 (%)
Age (yr) [*]	28.8±10.1	26.8±10.9	44.7±6.0	-
Male to Female [#]	102:32	34:1	3:0	1:0
Age at onset (yr)	21.4±8.4	19.6±10.3	29.0±12.0	-
Course of disease	7.4±7.1	6.9±6.8	15.7±16.3	-
Diagnostic delay	6.8±6.9	6.0±6.3	3.0±1.9	-
ESR (mm/h)	67.5±58.7	57.8±35.1	71±32.5	-
CRP (mg/L)	38.0±34.0	46.7±45.5	62.4±60.2	-
Radiographic sacroiliitis				
Grade 0	6 (4.5)	1 (2.9)	0	0
Grade I	9 (6.7)	3 (8.6)	0	0
Grade II	54 (40.3)	15 (42.9)	1 (33.3)	0
Grade III	47 (35.1)	12 (34.3)	1 (33.3)	1
Grade IV	18 (13.4)	4 (11.4)	1 (33.3)	0
Disease pattern				
Axial	59 (44.0)	13 (37.1)	2 (66.7)	0
Peripheral	70 (52.2)	19 (54.3)	1 (33.3)	1
Mixed	5 (3.7)	3 (8.6)	0	0
Family history of SpA [*]	39 (29.1)	7 (20.0)	3 (100.0)	0
Initial symptoms				
Spinal column involvement	64 (47.8)	16 (45.7)	2 (66.7)	0
Upper back pain	4 (3.0)	0	0	0
Lower back pain	49 (36.6)	12 (34.3)	2 (66.7)	0
Buttock pain	8 (6.0)	4 (11.4)	0	0
Peripheral arthritis	71 (53.0)	22 (62.9)	1 (33.3)	1 (100.0)
Hip	38 (28.4)	9 (25.7)	0	0
Shoulder	2/(1.5)	1 (2.9)	0	0
Heel pain	5 (3.7)	2 (5.7)	0	0
Other enthesitis	2 (1.5)	0	0	0
Extra-articular features [*]	9 (6.7)	6 (17.1)	2 (66.7)	0
Fever	2/9 (22.2)	2 (33.3)	1 (50.0)	0
Uveitis	1/9 (11.1)	3 (50.0)	0	0
Renal disease	1/9 (11.1)	1 (16.7)	0	0
Diagnosis categories				
AS/uSpA	118/15	31/4	3/0	1/0
JAS/AAS [§]	40/78	16/15	0/3	1/0

Quantitative data were presented as means±SD and qualitative data were described with ratios or percentages.

* $P<.05$, # $P=.01$, and § $P=.06$.

HLA-B*2704, B*2705, B*2707, and B*2724. Of them, HLA-B*2704 is the most common subtype in our Han Chinese,³⁸ followed by HLA-B*2705 which is a predominant subtype in Caucasians.³⁹ B*2707 and B*2724 were also reported in Xinjiang Uygur AS patients.³³ Collectively, these findings were consistent with several previously published studies.^{17,40}

Numerous studies have been conducted to investigate the crucial role of HLA-B*27 in susceptibility to SpA including AS.^{8,9,16,18} Indeed, relatively few studies performed the profound and prudent exploration of its effects on disease expression or clinical severity of SpA patients.^{37,39,41} In the current study, a noticeably male predominance and markedly elevated CRP were found in B*27 positive SpA patients.

In multivariable linear regression analysis, the elevated CRP was significantly associated with HLA-B*27 positivity, grade of sacroiliitis, and male gender. This result was in agreement with Liao et al.,⁴² but was contrary to the report from Chung et al.⁴³ The reasons for these discrepancies may not only be methodological, but may also reflect different treatment efficacy of nonsteroidal anti-inflammatory drugs, at least to some degree.

In order to investigate the subtle differences between HLA-B*27 subtypes, B*27 positive patients were stratified according to their HLA-B*27 alleles. Notably, there was also a sharply male predilection in B*2705 positive SpA patients. Contrary to those previous studies which reported that onset age of B*27 positive SpA patients was much younger than B*27 negative counterparts,^{44,45} our study could not verify such an association between different groups. Similarly, a recent study did not find significant differences in onset age between B*2704 positive and B*27 negative Chinese JAS patients.³⁷ However, the present study found that a higher percentage of JAS was observed in B*2705 than that in B*2704 and B*2707 positive patients, which suggested relatively more B*2705 positive patients would be with an early onset of disease. Interestingly, more familial aggregation and extra-articular features were observed in B*2707 positive SpA patients. These observations are inconsistent with the view that the HLA-B*2707 subtype was a possibly protective allele for incidence of SpA.⁴⁶ However, quite a few studies coming from different populations, in fact, have also reported B*2707 positive patients.^{47,48} Due to its relative rarity in our population, the right and eligible conclusion about this subtype need to be confirmed in a larger number of SpA patients.

In conclusion, our study developed a set of primers mixture for high-resolution HLA-B*27 subtyping and assessed the role of HLA-B*27 exerting in clinical manifestations of SpA in Han Chinese. We highlighted that a significant male predilection and elevated CRP were found in B*27 positive SpA patients. What is more, higher prevalence of extra-articular features and familial aggregation were demonstrated in HLA-B*2707 positive SpA patients, while, the male predominance and relative much more percent of JAS were seen in HLA-B*2705 positive SpA patients. These findings may improve our knowledge on the underlying genetic contributions of HLA-B*27 polymorphisms to phenotypic differences among Chinese patients with SpA.

ACKNOWLEDGMENTS

The authors thank all patients and their families who took part in the study. This work was funded by research project from Xinxiang Medical University (2007YJA07). The authors declare no conflicts of interest.

REFERENCES

- van Tubergen A, Weber U. Diagnosis and classification in spondyloarthritis: identifying a chameleon. *Nat Rev Rheumatol*. 2012;8:253-261.
- Brewerton DA, Hart FD, Nicholls A, et al. Ankylosing spondylitis and HL-A 27. *Lancet*. 1973;1:904-907.
- Marcilla M, Lopez de Castro JA. Peptides: the cornerstone of HLA-B27 biology and pathogenetic role in spondyloarthritis. *Tissue Antigens*. 2008;71:495-506.
- van den Berg R, de Hooge M, van Gaalen F, et al. Percentage of patients with spondyloarthritis in patients referred because of chronic back pain and performance of classification criteria: experience from the Spondyloarthritis Caught Early (SPACE) cohort. *Rheumatology (Oxford)*. 2013;52:1492-1499.
- Bemtson L, Nardal E, Aalto K, et al. HLA-B27 predicts a more chronic disease course in an 8-year Followup Cohort of Patients with Juvenile Idiopathic Arthritis. *J Rheumatol*. 2013;40:725-731.
- Rudwaleit M, van der Heijde D, Khan MA, Braun J, Sieper J. How to diagnose axial spondyloarthritis early. *Ann Rheum Dis*. 2004;63:535-543.
- Cheung PP, Paternotte S, Burki V, et al. Performance of the assessment in Spondyloarthritis International Society classification for axial and peripheral spondyloarthritis in an established clinical cohort: comparison with criteria sets of Amor and the European Spondylarthropathy Study Group. *J Rheumatol*. 2012;39:816-821.
- Breban M, Miceli-Richard C, Zinovieva E, Monnet D, Said-Nahal R. The genetics of spondyloarthropathies. *Joint Bone Spine*. 2006;73:355-362.
- Chatzikyriakidou A, Voulgari PV, Drosos AA. What is the role of HLA-B27 in spondyloarthropathies? *Autoimmun Rev*. 2011;10:464-468.
- Hammer RE, Maika SD, Richardson JA, Tang JP, Taurog JD. Spontaneous inflammatory disease in transgenic rats expressing HLA-B27 and human beta 2 m: an animal model of HLA-B27-associated human disorders. *Cell*. 1990;63:1099-1112.
- Cauli A, Dessole G, Fiorillo MT, et al. Increased level of HLA-B27 expression in ankylosing spondylitis patients compared with healthy HLA-B27-positive subjects: a possible further susceptibility factor for the development of disease. *Rheumatology (Oxford)*. 2002;41:1375-1379.
- Evans DM, Spencer CC, Pointon JJ, et al. Interaction between ERAP1 and HLA-B27 in ankylosing spondylitis implicates peptide handling in the mechanism for HLA-B27 in disease susceptibility. *Nat Genet*. 2011;43:761-767.
- Akram A, Lin A, Gracey E, Streutker CJ, Inman RD. HLA-B27, but not HLA-B7, immunodominance to influenza is ERAP dependent. *J Immunol*. 2014;192:5520-5528.
- Tran TM, Hong S, Edwan JH, Colbert RA. ERAP1 reduces accumulation of aberrant and disulfide-linked forms of HLA-B27 on the cell surface. *Mol Immunol*. 2016;74:10-17.
- Mou Y, Wu Z, Gu J, et al. HLA-B27 polymorphism in patients with juvenile and adult-onset ankylosing spondylitis in Southern China. *Tissue Antigens*. 2010;75:56-60.
- Dougados M, Baeten D. Spondyloarthritis. *Lancet*. 2011;377:2127-2137.
- Duangchanchot M, Puapairoj C, Romphruk A, et al. HLA-B*27 subtypes in Northern and Northeastern Thais, Karens, and Bamars determined by a high-resolution PCR-SSP technique. *Tissue Antigens*. 2009;73:590-594.
- Khan MA. Polymorphism of HLA-B27: 105 subtypes currently known. *Curr Rheumatol Rep*. 2013;15:362.
- van der Linden S, Valkenburg HA, Cats A. Evaluation of diagnostic criteria for ankylosing spondylitis. A proposal for modification of the New York criteria. *Arthritis Rheum*. 1984;27:361-368.
- Dougados M, van der Linden S, Juhlin R, et al. The European Spondylarthropathy Study Group preliminary criteria for the classification of spondylarthropathy. *Arthritis Rheum*. 1991;34:1218-1227.
- Sieper J, Rudwaleit M, Baraliakos X, et al. The Assessment of SpondyloArthritis international Society (ASAS) handbook: a guide to assess spondyloarthritis. *Ann Rheum Dis*. 2009;68(Suppl 2):ii1-ii44.
- van Tubergen A, Heuft-Dorenbosch L, Schulpen G, et al. Radiographic assessment of sacroiliitis by radiologists and rheumatologists: does training improve quality? *Ann Rheum Dis*. 2003;62:519-525.

23. Olerup O. HLA-B27 typing by a group-specific PCR amplification. *Tissue Antigens*. 1994;43:253-256.
24. Zino E, Di Terlizzi S, Carugo C, et al. Rapid detection of all HLA-B*27 alleles (B*2701-B*2725) by group-specific polymerase chain reaction. *Tissue Antigens*. 2004;63:88-92.
25. Ma HJ, Hu FP. Diversity of human leukocyte antigen-B27 alleles in Han population of Hunan province, southern China. *Tissue Antigens*. 2006;68:163-166.
26. Kirveskari J, Kellner H, Wuorela M, et al. False-negative serological HLA-B27 typing results may be due to altered antigenic epitopes and can be detected by polymerase chain reaction. *Brit J Rheumatol*. 1997;36:185-189.
27. Huang F, Yamaguchi A, Tsuchiya N, et al. Induction of alternative splicing of HLA-B27 by bacterial invasion. *Arthritis Rheum*. 1997;40:694-703.
28. Seipp MT, Erali M, Wies RL, Wittwer C. HLA-B27 typing: evaluation of an allele-specific PCR melting assay and two flow cytometric antigen assays. *Cytometry B Clin Cytom*. 2005;63:10-15.
29. Nieto A, Fraile A, Vinasco J, Martin J. HLA-B*27 typing by PCR-restriction fragment length polymorphism. *Tissue Antigens*. 1997;49:283-286.
30. Martinez-Borra J, Gonzalez S, Lopez-Vazquez A, et al. HLA-B27 alone rather than B27-related class I haplotypes contributes to ankylosing spondylitis susceptibility. *Hum Immunol*. 2000;61:131-139.
31. Brown MA, Pile KD, Kennedy LG, et al. HLA class I associations of ankylosing spondylitis in the white population in the united Kingdom. *Ann Rheum Dis*. 1996;55:268-270.
32. Acar M, Cora T, Tunc R, Acar H. HLA-B27 subtypes in Turkish patients with ankylosing spondylitis and healthy controls. *Rheumatol Int*. 2012;32:3103-3105.
33. Zou HY, Yu WZ, Wang Z, He J, Jiao M. Human leukocyte antigen-B27 alleles in Xinjiang Uygur patients with ankylosing spondylitis. *Genet Mol Res*. 2015;14:5652-5657.
34. Downing J, Coates E, Street J, et al. A high-resolution polymerase chain reaction-sequence-specific primer HLA-B*27 typing set and its application in routine HLA-B27 testing. *Genet Test*. 2006;10:98-103.
35. Tomero E, Mulero J, de Miguel E, et al. Performance of the Assessment of Spondyloarthritis International Society criteria for the classification of spondyloarthritis in early spondyloarthritis clinics participating in the ESPERANZA programme. *Rheumatology (Oxford)*. 2014;53:353-360.
36. Casals-Sanchez JL, Garcia De Yebenes Prous MJ, Descalzo Gallego MA, et al. Characteristics of patients with spondyloarthritis followed in rheumatology units in Spain. emAR II study. *Reumatol Clin*. 2012;8:107-113.
37. Mou Y, Zhang P, Li Q, et al. Clinical features in Juvenile-onset ankylosing spondylitis patients carrying different B27 subtypes. *Biomed Res Int*. 2015;2015:594878.
38. Liu Y, Jiang L, Cai Q, et al. Predominant association of HLA-B*2704 with ankylosing spondylitis in Chinese Han patients. *Tissue Antigens*. 2010;75:61-64.
39. Johnsen SS, Bakland G, Nossent JC. The distribution of HLA-B27 subtype in patients with ankylosing spondylitis in Northern Norway. *Scand J Rheumatol*. 2014;43:296-300.
40. Chou CT, Chen JM, Hsu CM, Chen SJ. HLA-B27 and its subtypes in 4 Taiwanese Aborigine tribes: a comparison to Han Chinese patients with ankylosing spondylitis. *J Rheumatol*. 2003;30:321-325.
41. Chavan H, Samant R, Deshpande A, Mankeshwar R. Correlation of HLA B27 subtypes with clinical features of ankylosing spondylitis. *Int J Rheum Dis*. 2011;14:369-374.
42. Liao Z, Lin Z, Xu M, et al. Clinical features of axial undifferentiated spondyloarthritis (USpA) in China: HLA-B27 is more useful for classification than MRI of the sacroiliac joint. *Scand J Rheumatol*. 2011;40:439-443.
43. Chung HY, Machado P, van der Heijde D, D'Agostino MA, Dougados M. HLA-B27 positive patients differ from HLA-B27 negative patients in clinical presentation and imaging: results from the DESIR cohort of patients with recent onset axial spondyloarthritis. *Ann Rheum Dis*. 2011;70:1930-1936.
44. Feldtkeller E, Khan MA, van der Heijde D, van der Linden S, Braun J. Age at disease onset and diagnosis delay in HLA-B27 negative vs. positive patients with ankylosing spondylitis. *Rheumatol Int*. 2003;23:61-66.
45. Rudwaleit M, Haibel H, Baraliakos X, et al. The early disease stage in axial spondylarthritis results from the German spondyloarthritis inception cohort. *Arthritis Rheum*. 2009;60:717-727.
46. Varnavidou-Nicolaidou A, Karpasitou K, Georgiou D, et al. HLA-B27 in the Greek Cypriot population: distribution of subtypes in patients with ankylosing spondylitis and other HLA-B27-related diseases. The possible protective role of B*2707. *Hum Immunol*. 2004;65:1451-1454.
47. Zambrano-Zaragoza JF, Agraz-Cibrian JM, Gonzalez-Reyes C, Duran-Avelar MdeJ, Vibanco-Perez N. Ankylosing spondylitis: from cells to genes. *Int J Inflam*. 2013;2013:501653.
48. Yi L, Wang J, Guo X, et al. Profiling of hla-B alleles for association studies with ankylosing spondylitis in the Chinese population. *Open Rheumatol J*. 2013;7:51-54.

How to cite this article: Ma H-J, Yin Q-F, Liu Y, Wu Y, Zhu T-C, Guo M-H. Polymorphisms of human leukocyte antigen B*27 on clinical phenotype of spondyloarthritis in Chinese. *J Clin Lab Anal*. 2018;32:e22275. <https://doi.org/10.1002/jcla.22275>