Profiling of HLA-B Alleles for Association Studies with Ankylosing Spondylitis in the Chinese Population

Lin Yi[1,2,§], Jiucun Wang[3,5,§], Xinjian Guo[1], Maribel G. Espitia[1], Enuo Chen[1], Shervin Assassi[1],
Li Jin[3], Hejian Zou[*,4,5], John D. Reveille[1] and Xiaodong Zhou[*,1]

[1]*Division of Rheumatology and Clinical Immunogenetics, University of Texas Medical School at Houston, USA*

[2]*Gansu College of Traditional Chinese Medicine, Lanzhou, Gansu, China*

[3]*State Key Laboratory of Genetic Engineering and Ministry of Education Key Laboratory of Contemporary Anthropology, School of Life Sciences, Fudan University, China*

[4]*Huashan Hospital, Fudan University, China*

[5]*Institute of Rheumatology, Immunology, and Allergy, Fudan University, China*

Abstract: Human leucocyte antigen (HLA) B*27 is a susceptibility allele to ankylosing spondylitis (AS). However, major AS-associated subtypes of HLA-B*27 and other HLA-B alleles vary in different ethnic populations. Herein, we examined HLA-B alleles in a total of 360 AS patients and 350 controls of Chinese Han ancestry. The HLA-B genotyping was performed with sequence-based typing (SBT) method. Six HLA-B*27 subtypes B*27:04, B*27:05, B*27:07, B*27:08, B*27:10 and B*27:15 were observed in the cohorts. HLA-B*27:04:01 and -B*27:05:02 appeared significantly increased in AS patients, which indicated as two major susceptibility alleles to AS. Homozygous B*27 was observed only in AS patients. There are 30 HLA-B alleles identified in the studies. HLA-B*15, especially B*15:01:01:01, appeared as the major allele type in the Chinese controls. Some common HLA-B alleles such as HLA-B*15, B*13, B*46 and B*51 were significantly reduced in Chinese AS patients. In conclusion, the studies profiled the HLA-B alleles, and identified major susceptibility subtypes of B27 to AS in Han Chinese population.

Keywords: Ankylosing spondylitis (AS), HLA-B27, Chinese Han.

INTRODUCTION

Ankylosing spondylitis (AS) is an immune-mediated chronic disease characterized by inflammation of the axial skeleton, as well as extraspinal involvement. Although, etiopathogenesis is not fully understood, genetic factors play a key role in AS susceptibility. The human leukocyte antigen (HLA) class I molecules are essential in the immune regulation, especially in defense against intracellular infections (e.g. viruses). In fact, representing the wide array of infectious challenges facing humans, HLA alleles have evolved as the most polymorphic loci in the genome, with over 6919 MHC class I and 1875 class II alleles having been reported as of January, 2013 (http://www.ebi.ac.uk/ipd/imgt/hla/stats.html), many of which conferring susceptibility to specific immune-mediated diseases.

*Address correspondence to these authors at the Division of Rheumatology and Clinical Immunogenetics, University of Texas Medical School at Houston, 6431 Fannin Street, Room 5.270 Houston, Texas 77030, USA; Tel: 713-500-6900; Fax: 713-500-0580;
E-mail:xiaodong.zhou@uth.tmc.edu and Huashan Hospital, Fudan University, China; Tel: +86-21-52888808; Fax: +86-21-62489191; E-mail: hejianzou@gmail.com

§First two authors contributed to the studies equally.

HLA-B is a class I molecule, and HLA-B27 is a well-documented genetic risk allele for ankylosing spondylitis (AS) in all examined populations, which contributes 23.3% of AS heritability [1]. However, of the over 100 B*27 alleles reported to date, the major AS-associated subtypes of HLA-B27 vary in frequency in different ethnic populations. HLA-B*27:02 was reported in AS patients of Europeans, especially more frequent in Southern European [2]. HLA-B*27:03 appeared in Black African populations [3]. HLA-B*27:04 is the most common AS-risk allele in Chinese Han, Taiwanese and Japanese [4-6]. HLA-B*27:05, the "parent" HLA-B27 allele, is present in almost all populations, and is the major AS allele in Caucasians, American Indians [3] and Koreans [7]. HLA-B*27:06 is a relatively rare subtype of HLA-B27 occurring most commonly in Southeast Asian individuals, Thai, Taiwanese and Singaporean populations [8], and is thought to be unassociated with AS. Similarly, the presence of HLA-B*27:07 and HLA-B*27:08 were reported less frequently in AS patients. HLA-B*27:07 may play a protective role [9]. HLA-B*27:09 described only in Sardinia and southern Italy [10], and has not been observed to data in AS patients.

Although, several studies of HLA-B have been reported in Chinese AS patients, most of them examined only B*27 positive individuals. B*27 negative information is generally lack in the studies. Recently, we established an AS cohort

and controls of Han Chinese. Our studies aimed to profile the HLA-B alleles, and to determine major and minor HLA-B alleles, as well as whether they are potential risk or protective alleles to AS in Han Chinese.

MATERIALS AND METHODOLOGY

Patients and Controls

Han Chinese patients and controls were enrolled from the clinics and hospitals in Shanghai and Jiangsu Province of China. A total of 360 AS patients and 350 controls of Chinese Han ancestry were examined in the studies. All patients met the modified New York criteria for this disease [11]. All participants underwent a clinical evaluation by one of the study rheumatologists, had pelvic and spinal radiographs to confirm their diagnosis, and completed questionnaires about their personal and medical history and functional status. Unrelated controls were free of any history of rheumatic disease. Chinese controls were obtained from a study project of Chinese population genetics in Fudan University, Shanghai, China. All subjects signed the informed consent. The studies were approved by Ethic Committee of both Fudan University and the University of Texas Medical School at Houston.

Sequencing Genotyping

Genomic DNA was extracted from peripheral blood cells from subjects. The HLA-B genotyping was performed with sequence-based typing (SBT) method using SeCore Kits (Life Technologies, USA). Briefly, the allele-specific polymerase chain reactions (PCR) were performed using primers supplied in the SeCore kits, and then were followed by sequencing exon 2 and 3 of the HLA-B gene. The HLA SBT uTYPE 6.0 program (Life Technologies) was used in sequencing analysis and assigning HLA-B alleles.

Data Analysis

Tests of association between genetic variants and AS were performed for each allele using Epi Info program developed by the Center for Disease Controls and Prevention (CDC. Atlanta, Georgia, USA). Exact p-values were obtained (Fisher's test) from 2 x 2 tables of allele counts and disease status.

RESULTS

Sequencing data were first filtered with quality control according to the HLA SBT uTYPE 6.0 program. The samples that failed in sequencing were re-examined. There were six AS patients and eight controls who failed in genotyping although the sequencing quality passed the filter. A total of 354 AS patients and 342 controls were successfully genotyped.

There were thirty HLA-B alleles identified in the studies (Table **1**). HLA-B*15, especially B*15:01:01:01, appeared as the most common allele in controls (31.3% controls carrying B*15 with allele frequency of 17.5%). HLA-B*40, HLA-B*13 and HLA-B*46 also appeared as common alleles of Chinese controls with corresponding allele frequencies of 12.8%, 11.4% and 10% respectively (Table **1**). Statistical results for each of HLA-B alleles are listed in the Table **1**.

Table 1. Allele Frequency of HLA-B in Han Chinese AS Cases and Controls

Alleles	AS	%	Control	%	p	OR
B*07	1	0.1	12	1.8	0.0018	0.08
B*08	2	0.3	5	0.7	0.237	0.38
B*13	39	5.5	78	11.4	7.4×10^{-5}	0.45
B*14	3	0.4	1	0.2	0.334	2.91
B*15	51	7.2	120	17.5	$<1 \times 10^{-7}$	0.36
B*18	2	0.3	1	0.2	0.584	1.93
B*27	336	47.5	26	3.8	$<1 \times 10^{-7}$	22.86
B*35	14	2.0	37	5.4	6.6×10^{-4}	0.35
B*37	2	0.3	4	0.6	0.389	0.48
B*38	12	1.7	20	2.9	0.126	0.57
B*39	15	2.1	10	1.5	0.356	1.46
B*40	73	10.3	88	12.9	0.136	0.78
B*41	0	0	1	0.1	0.309	0
B*44	9	1.3	16	2.3	0.134	0.54
B*45	0	0	7	1.0	0.007	0
B*46	36	5.1	68	10	5.7×10^{-4}	0.49
B*48	12	1.7	11	1.6	0.899	1.05
B*50	2	0.3	0	0	0.164	NA
B*51	29	4.1	60	8.8	3.6×10^{-4}	0.44
B*52	12	1.7	19	2.8	0.171	0.6
B*53	1	0.1	0	0	0.325	NA
B*54	10	1.4	19	2.8	0.075	0.5
B*55	12	1.7	14	2.1	0.628	0.83
B*56	1	0.1	4	0.6	0.167	0.24
B*57	4	0.6	4	0.6	0.96	0.97
B*58	25	3.5	50	7.3	0.0018	0.46
B*59	0	0	4	0.6	0.042	0
B*67	3	0.4	3	0.4	0.966	0.97
B*78	1	0.1	2	0.3	0.543	0.48
B*81	1	0.1	0	0	0.325	NA

A *p*-value less than 0.00167 is significant after correction for multiple comparison.

HLA-B*27 occurred in 93% in AS patients and 7.6% of Han Chinese controls. There were six subtypes of the HLA-B*27 (B*27:04, B*27:05, B*27:07, B*27:08, B*27:10 and B*27:15) observed in Chinese AS patients, only three of them (B*27:04, B*27:05 and B*27:07) occurred in the controls (Table **2**). Among the six subtypes, B*27:04 (or B*27:04:01) appeared in 68.1% AS patients versus in 4.1% controls, and B*27:05:02 in 22% AS patients versus 2.63% controls. HLA-B*27:07:01, B*27:08, B*27:10 and B*27:15 were sporadically observed in the AS patients. There were nine AS patients homozygous for B*27 alleles, of whom four were homozygous for B*27:04:01. In contrast, all controls with B*27 positive were heterozygous with a non-

B*27 allele (p = 0.0033). Specific allele frequencies in cases and controls and their statistical results are listed in the Table **2**.

Table 2.　Presence of the HLA-B27 Subtypes (Allele Frequency) in All Chinese AS Patients and Controls

Alleles	AS	%	Control	%	p	OR
B*27:04						
B*27:04:01	245	34.6	16	2.3	$<1 \times 10^{-7}$	22.09
B*27:05						
B*27:05:01	1	0.1	0	0	0.325	NA
B*27:05:02	79	11.2	9	1.3	$<1 \times 10^{-7}$	9.42
B*27:07	5	0.7	1	0.2	0.111	4.86
B*27:08	1	0.1	0	0	0.325	NA
B*27:10	1	0.1	0	0	0.325	NA
B*27:15	4	0.6	0	0	0.049	NA
Total B alleles	708		684			

DISCUSSION

The genetic association of HLA-B*27 with AS was discovered first in the early 1970's, and was persistently confirmed in later studies. However, specific subtypes of HLA-B27 and major susceptibility alleles of the subtypes to AS vary in different ethnic populations. Specific alleles and subtypes of HLA genes are named according to their corresponding serologic antigen and the complexity of gene sequence. In general, the first set of digits (first two digits) corresponds to the serological antigen carried by an allotype. The second set of digits (second two digits) is used to name the subtypes that are nonsynonymous nucleotide substitutions. The third set of digits (third two digits) is assigned to synonymous nucleotide substitutions, the fourth set of digits (fourth two digits) indicate specific sequence polymorphisms in non-coding regions of the gene.

In Chinese population, HLA-B*27:04 was reported as the major susceptibility allele in AS patients, and was followed by HLA-B*27:05 [12-14]. In addition to these two subtypes, B*27:02, B*27:03, B*27:06, B*27:10, B*27:13, B*27:15 and B27:24 also were reported in Chinese AS patients. The results of our studies indicated that HLA-B*27:04 primarily and B*27:05 secondarily were the most highly associated AS alleles, consistent with previous reports. Our results specified these two alleles of subtypes with the third set of digits as B*27:04:01 and B*27:05:02. In addition to these two alleles, B*27:07, B*27:08, B*27:10 and B*27:15 also were observed in our AS patients. Among them, B*27:07 (B*27:07:01) and B*27:08 are reported first time in Chinese AS patients, although their allele frequencies were only 0.7% and 0.1%, respectively (Table **2**). However, a protective role of B*27:07 against AS that was reported previously [9] cannot be confirmed here. A larger sample size of studies may be necessary to clarify this association. Another important feature of this report is that homozygous B*27 was observed in 2.54% AS patients, but was absent in controls, which supports a risk role of B*27 to Chinese AS.

In contrast to HLA-B*27 as the major allele in Chinese AS patients, HLA-B*15 was observed in 31.3% Chinese controls as the most common allele (allele frequency of 17.5%). Specifically, B*15:01:01:01 was the major subtype of B*15 in the population. HLA-B*13, HLA-B*40 and HLA-B*46 also appeared as common alleles of Chinese controls (allele frequency ≥10%) (Table **1**). HLA-B*13, B*15 and B*46 were significantly decreased in the patients indicating their potential protective role against AS of Chinese population. There were a total of 30 HLA-B alleles identified in the studies, which provided a profile of Chinese HLA-B alleles.

In addition to HLA-B*27 with AS, HLA-B*14:03 also was associated with AS in a West African population [15], however, it was not observed in Chinese population in our studies. It is worth noting that various HLA-B alleles have been implicated in association with other disease susceptibility and clinical outcomes. For instance, HLA-B*51 has been recognized as genetic susceptibility factor for Behcet's disease (BD) [16], HLA-B*44 was reported to protect against multiple sclerosis (MS) [17], HLA-B*58:01 and B*15:02 were associated with allopurinol-induced and carbamazepine-induced Stevens-Johnson syndrome and toxic epidermal necrolysis, respectively [18-20], HLA-B*57:01 was considered as a major determinant of drug-induced liver injury due to flucloxacillin [21]. Our profiling of HLA-B alleles of Chinese population provides a reference for future studies of different diseases in association with specific HLA-B alleles.

CONCLUSION

Our studies profiles the HLA-B alleles, and identified HLA-B*27:04:01 and B*27:05:02 as two major susceptibility suballeles of HLA-B*27 to AS in Han Chinese population. We also demonstrated that homozygous HLA-B*27 only occurred in AS patients which may suggest strong risk to AS. Some common alleles of Chinese population including B*15, B*13 and B*46 are significantly reduced in Chinese AS patients, which may implicate potentially protective role to AS.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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