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ASSOCIATION OF HLA-DQB1*0501 WITH SCLERODERMA AND ITS CLINICAL FEATURES IN CHINESE POPULATION

X.D. ZHOU[1], **L. YI**[1,2], **X.J. GUO**[1], **E. CHEN**[1], **H.J. ZOU**[3,4], **L. JIN**[5], **M.D. MAYES**[1], **S. ASSASSI**[1], and **J.C. WANG**[4,5]

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

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

[3]Huashan Hospital, Fudan University, Shanghai, China

[4]Institute of Rheumatology, Immunology, and Allergy, Fudan University, Shanghai, China

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

Abstract

Specific human leukocyte antigen (HLA) DQB1 alleles confer strong susceptibility to systemic sclerosis (SSc). However, the frequencies of specific DQB1 alleles and their associations with SSc vary according to ethnicity and clinical features of SSc. The aim of this study was to profile DQB1 alleles in a Chinese population and to identify specific DQB1 alleles in association with SSc of Han Chinese. A cohort containing 213 patients with SSc and 239 gender-matched and unrelated controls was examined in the study. The HLA-DQB1 genotyping was performed with sequence-based typing (SBT) method. Exact p-values were obtained (Fisher's test) from 2×2 tables of allele counts or allele carriers and disease status. Seventeen DQB1 alleles were found in the cohort. DQB1*03:03 was the most common allele in this cohort. DQB1*05:01 was significantly increased in SSc, and was strongly associated with anticentromere autoantibodies (ACA). Compared with SSc in other ethnic populations, SSc patients of Han Chinese are distinct in association with DQB1*06:11, common in association with DQB1*05:01, but lack association with DQB1*03:01. In addition, DQB1*06:01 appeared more common in ATA-positive Chinese SSc, and marginally associated with pulmonary fibrosis, and an increased frequency of DQB1*03:03 was observed in anti-U1 RNP-positive Chinese SSc patients.

Keywords

scleroderma; HLA; genetic association; susceptibility; Chinese population

Systemic sclerosis (SSc) or scleroderma is a rare and complex autoimmune disease. It is clinically classified into two subsets: limited cutaneous (lcSSc) and diffuse cutaneous (dcSSc) SSc. The latter subset is characterized by more rapid progression of skin and visceral involvement, as well as poorer prognosis (1). SSc also can be subgrouped by

Mailing address: Dr Xiaodong Zhou, Division of Rheumatology and Clinical Immunogenetics, University of Texas Medical School, 6431 Fannin Street, Room 5.270 Houston, Texas 77030, USA, Tel.: +1 713 500 6900 Fax: +1 713 500 0580, xiaodong.zhou@uth.tmc.edu.

DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

autoantibody subsets. The most common of these autoantibodies are directed against DNA topoisomerase I (ATA), centromeric proteins (ACA) and RNA polymerases III (anti-RNAP3), as well as other less commonly occurring autoantibodies including U1-ribonucleoprotein (anti-U1RNP) (2, 3).

Specific human leukocyte antigen (HLA) DQB alleles have been associated with SSc. However, major SSc-associated HLA-DQB alleles vary in different ethnic populations. For instance, DQB1*03:01 was associated with susceptibility to SSc in US Caucasian, Hispanic and African-American populations (4), but not in Koreans (5) and only with dcSSc in South Africans (6); DQB1*02:02 and *06:02 were associated with protection from SSc in only US Caucasian, DQB1*05:01 allele was significantly increased in US Caucasian, Spanish and Japanese patients with ACA (4, 7, 8), but not in Hispanic and African-Americans (4).

Chinese SSc patients have unique serological and clinical features with high frequency of ATA, dcSSc and pulmonary fibrosis but low in anti-RNAP3 (9). Associations between the HLA alleles and SSc have not been reported in Chinese SSc. Recently, we established an SSc cohort of Han Chinese through multicenter SSc consortium in China under the International Network of Scleroderma Clinical Care and Research (InSCAR) (http://www.inscar-global.org). The goal of the present study is to investigate the HLA-DQB1 alleles in association with potential risk to or protection from SSc in Han Chinese.

A total of 213 patients with SSc and 239 gender-matched and unrelated controls were examined for HLA-DQ1 alleles in the studies. SSc patients were recruited from a multicenter study including hospitals and outpatient clinics in Shanghai, Hebei province, Sichuan province, and Hunan province in China. All patients met the American College of Rheumatology (ACR) classification criteria for SSc (10), or had at least 3 out of 5 CREST features (Calcinosis, Raynaud's phenomenon, Esophageal dysmotility, Sclerodactyly, and Telangiectasia) with sclerodactyly being mandatory (11). None of the controls had autoimmune diseases. SSc patients were 91.1% positive for ANA. There were 79 lcSSc and 98 dcSSc, others were undefined. One hundred and ninety patients were examined for ATA with 84 being positive; 163 were examined for ACA with 23 positive; 156 were examined for anti-RNAP3 with 4 positive; 182 were examined for anti-U1RNP with 34 positive. Out of 176 patients who were examined with chest X-ray and/or CT, 134 were diagnosed with positive pulmonary fibrosis. Patients' sera were tested for antinuclear antibodies (ANA) by indirect immunofluorescence using HEp-2 cells as antigen substrate (Antibodies, Davis, CA, USA). ATA and anti-U1RNP were detected by passive immunodiffusion against calf thymus extracts (INOVA, Diagnostics). ACA was determined by indirect immunofluorescence using HEp-2 cells. Anti-RNAP3 was detected utilizing commercially available kits (NBL, Nagoya, Japan). Genomic DNA was extracted from peripheral blood cells from subjects. The HLA-DQB1 genotyping was performed with sequence-based typing (SBT) method using SeCore Kits (Life Technologies, USA). The HLA SBT uTYPE 6.0 program (Life Technologies) was used in sequencing analysis and assigning HLA-DQB1 alleles. Exact p-values were obtained (Fisher's test) from 2×2 tables of allele counts and disease status. The statistical p values less than 0.05 were considered passing normal significance level. Correction for multiple comparisons was performed according to Bonferroni's method. Specifically, a p-value less than 0.00294 was considered significant after adjustment for 17 comparisons.

A total of seventeen DQB1 alleles were found in the cohort. Frequency of each allele in SSc patients and controls are listed in Table I. HLA-DQB1*03 alleles appeared the most common, especially DQB1*03:03 in 30.1% of controls *vs* in 25.6% of SSc patients, and DQB1*03:01 in 23% controls *vs* in 23.2% SSc patients. In contrast to US Caucasians (4), the difference of allele frequencies of DQB1*03 alleles between Chinese SSc patients and

controls appeared not statistically significant. The lack of association between Chinese SSc and DQB1*03:01 was not unexpected. Previous studies of Korean and Japanese SSc also did not report any risk association of DQB1*03:01 with SSc (5, 12). Genetic heterogeneity among Asian and US populations may significantly impact the complex trait of SSc. DQB1*03:01 appeared to be one of the major DQB1 alleles in Han Chinese with 23% frequency in controls, in contrast to in only 16.8% of US Caucasians (4). It is worth noting that distribution of DQB1 alleles in the Chinese controls observed in our studies is similar to a previous report of a Chinese population (13).

On the other hand, DQB1*05:01 was significantly increased in SSc patients ($p = 1.6 \times 10^{-5}$, 10.6% in SSc *vs* 3.3% in controls in allele frequency, or 20.2% carriers in SSc patients *vs* 6.7% in controls), and DQB1*06:11 was only observed in SSc patients (1.2%, $p = 0.0163$) (Table I). Moreover, comparisons between SSc subsets and controls indicated that the DQB1*05:01 carriers were significantly increased in ACA positive SSc patients, in which 43.5% of patients carry this allele *vs* only 6.7% of controls ($p < 10^{-7}$, Odds ratio (OR) = 10.7), A significantly increased DQB1*05:01 also was observed with SSc patients with ATA (21.4%, $p = 1.5 \times 10^{-4}$, OR = 3.8) or pulmonary fibrosis (26.5%, $p = 6 \times 10^{-7}$, OR = 5.03) and with dcSSc (21.6%, $p = 2.1 \times 10^{-5}$ OR = 3.85).

However, comparisons between SSc subsets and controls may not clearly distinguish the association of the alleles with specific subsets of SSc from the association of the alleles with SSc disease in general. A comparison between subsets with and without a specific phenotype may be better to reveal genetic contribution to specific subsets of SSc. Such comparisons indicated that DQB1*05:01 was persistently associated with ACA positive SSc (Table II). This association is consistent with the observations in Caucasian, Spanish and Japanese SSc patients (4, 7, 8). In addition, dcSSc, ATA positive SSc and pulmonary fibrosis of SSc of Han Chinese also demonstrated a higher frequency of DQB1*05:01 (Table II), which was not reported in other ethnic populations.

In addition, DQB1*06:01 appeared more common in ATA positive Chinese SSc, and was marginally associated with pulmonary fibrosis (Table II), which may suggest its potential contribution to severity of SSc. Of note, an increased DQB1*06:01 frequency was also reported in Japanese SSc (14). Moreover, an increased frequency of DQB1*03:03 was observed in anti-U1RNP positive SSc patients, which was unique in this Han Chinese population, although the association was not persistent after Bonferroni's correction. These observations may need to be confirmed in a large sample size of SSc cohort and/or other ethnic populations.

Two previously reported SSc-protective alleles DQB1*02:02 and *06:02 in US Caucasians (4) did not show association with Chinese SSc, in which the former displayed a normal frequency, and the latter was slightly decreased. However, it appeared inconsistent with SSc of US Hispanics and African-Americans (4).

In summary, this is the first report of studies of HLA-DQB in Chinese SSc. It revealed different genetic aspects of SSc. The distribution of HLA-DQB1 alleles in Chinese SSc appeared largely different from other ethnic populations, especially Caucasians, Hispanics and Afro-Americans, which implicates ethnic differences in genetic association of SSc. On the other hand, significantly increased DQB1*05:01 in Chinese SSc patients also is a common risk allele to ACA positive SSc in several ethnic populations. Therefore, different ethnic populations also share some genetic determinants of SSC.

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Table I

Distribution of HLA-DQB1 alleles in Chinese controls and SSc patients.

Alleles	Control (%)	SSc (%)	p value	OR	95% CI
DQB1*02:01	16 (3.3)	21 (4.9)	NS		
DQB1*02:02	24 (5)	21 (4.9)	NS		
DQB1*03:01	110 (23)	99 (23.2)	NS		
DQB1*03:02	21 (4.4)	13 (13.1)	NS		
DQB1*03:03	144 (30.1)	109 (25.6)	NS		
DQB1*04:01	16 (3.3)	5 (1.2)	NS		
DQB1*04:02	7 (1.5)	6 (1.4)	NS		
DQB1*05:01	16 (3.3)	45 (10.6)	1.6×10^{-5}	3.4	1.8 - 6.4
DQB1*05:02	10 (2.1)	13 (3.1)	NS		
DQB1*05:03	20 (4.2)	16 (3.8)	NS		
DQB1*06:01	34 (7.1)	38 (8.9)	NS		
DQB1*06:02	31 (6.5)	19 (4.5)	NS		
DQB1*06:03	7 (1.5)	1 (0.2)	NS		
DQB1*06:04	9 (1.9)	6 (1.4)	NS		
DQB1*06:09	13 (2.7)	8 (1.9)	NS		
DQB1*06:10	0	1 (0.2)	NS		
DQB1*06:11	0	5 (1.2)	0.0163	NA	NA

NS : not significant. OR: odds ratio; CI: confidence interval; A p-value less than 0.00294 was considered significant after correction for multiple comparisons.

Table II

Associations between specific HLA-DQB1 alleles and subsets of Chinese SSc.

	DQB1*05:01			DQB1*06:01			DQB1*03:03		
SSc subsets	**n (%)**	**p**	**OR (95% CI)**	**n (%)**	**p**	**OR (95% CI)**	**n (%)**	**p**	**OR (95% CI)**
dcSSc	28 (14.3)	**0.048**	2.03	17 (8.7)	0.95	0.98	47 (24)	0.177	0.72
IcSSc	12 (7.6)		(0.95-4.4)	14 (8.9)		(0.44-2.18)	48 (30.4)		(0.44-1.19)
ATA (+)	18 (10.7)	0.74	0.9	21 (12.5)	**0.031**	2.19	39 (23.2)	0.26	0.77
ATA (-)	25 (11.8)		(0.45-1.78)	13.(6.1)		(1.01-4.79)	60 (28.3)		(0.47-1.25)
ACA (+)	12 (26.1)	**0.00067**	3.6	5 (10.9)	0.73	1.19	10 (21.7)	0.386	0.72
ACA (-)	25 (8.9)		(1.54-8.33)	26 (9.3)		(0.38-3.51)	78 (27.9)		(0.32-1.6)
anti-U1RNP (+)	8 (11.8)	0.87	1.07	3 (4.4)	0.16	0.43	25 (36.8)	**0.02**	1.93
anti-U1RNP (-)	33 (11.1)		(0.43-2.57)	29 (9.7)		(0.1-1.53)	69 (23.2)		(0.06-3.51)
PF (+)	30 (11.2)	0.9	1.05	28 (10.5)	**0.052**	3.15	63 (23.5)	0.11	0.65
PF (-)	9 (10.7)		(0.45-2.5)	3 (3.6)		(0.88-13.4)	27 (32.1)		(0.37-1.15)

*PF: pulmonary fibrosis