Association of HLA Class II Genes with Systemic Sclerosis in Koreans

SUNG HA KANG, MYOUNG HEE PARK, EUN YOUNG SONG, SU JIN KANG, EUN BONG LEE, YEONG WOOK SONG, and FUJIO TAKEUCHI

ABSTRACT. ***Objective.*** To investigate HLA class II associations with systemic sclerosis (SSc) in Koreans according to anti-topoisomerase I (anti-topo I), anti-centromere antibody (ACA), and anti-U1 ribonucleoprotein (RNP) status.

Methods. HLA class II alleles (DRB1, DRB5, DQB1) were determined by DNA typing in 200 healthy control subjects and 74 patients with SSc: 35 anti-topo I positive, 3 ACA positive, and 19 anti-U1 RNP positive; among them were 34 diffuse and 40 limited subtypes, and 16 overlap syndrome.

Results. Anti-topo I positive SSc was strongly associated with DRB1*1502 compared with both controls (23% vs 5%; $p_{corr} = 0.003$) and anti-topo I negative patients (23% vs 3%; $p = 0.009$); our study confirms observations in Japanese. HLA-DR ^{67}FLEDR^{71}, especially ^{38}V-^{67}FLEDR^{71} sequence (carried on DRB5*0102 in linkage disequilibrium with DRB1*1502, DRB1*0802, DRB1*1101), showed the strongest association with anti-topo I response (46% vs 16% in controls; $p_{corr} = 0.001$), and ^{38}L-^{67}FLEDR^{71} group alleles were not associated with anti-topo I response. Anti-topo I response was not significantly associated with HLA-DQB1 alleles in Koreans. There were only 3 ACA positive patients, and all patients had DRB1*1501 and DQB1*0602 as heterozygotes. Anti-U1 RNP occurred at a high frequency (63%) among patients with overlap syndrome, and was not associated with HLA-DR or DQ genes. Among anti-topo I negative patients, diffuse and limited subtypes of SSc were significantly associated with DRB1*0803 (47% vs 15% in controls; $p_{corr} < 0.05$) and DRB1*1501 (50% vs 17% in controls; $p_{corr} < 0.01$), respectively. These HLA associations have not been reported in other ethnic groups and possible associations with certain autoantibody subsets remain to be confirmed.

Conclusion. HLA-DR gene has a primary association with anti-topo I response, and HLA-DR ^{38}V-^{67}FLEDR^{71} group alleles including DRB5*0102 (in linkage disequilibrium with DRB1*1502) show the strongest association with anti-topo I response in Korean patients with SSc. (J Rheumatol 2001;28:1577–83)

Key Indexing Terms:
SYSTEMIC SCLEROSIS HLA-DR HLA-DQ KOREAN

From the Departments of Clinical Pathology and Internal Medicine, Seoul National University College of Medicine, Seoul; the Clinical Research Institute, Seoul National University Hospital, Seoul; the Department of Clinical Pathology, Hallym University College of Medicine, Chunchon, Korea; and the Department of Medicine, Faculty of Medicine, University of Tokyo, Tokyo, Japan.

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S.H. Kang, MD, Associate Professor of Clinical Pathology, Department of Clinical Pathology, Hallym University College of Medicine; M.H. Park, MD, Professor of Clinical Pathology, Department of Clinical Pathology, Seoul National University College of Medicine and Clinical Research Institute, Seoul National University Hospital; E.Y. Song, MD, Department of Clinical Pathology, Seoul National University College of Medicine; S.J. Kang, MS, Clinical Research Institute, Seoul National University Hospital; E.B. Lee, MD, Assistant Professor of Medicine; Y.W. Song, MD, Associate Professor of Medicine, Department of Internal Medicine, Seoul National University College of Medicine; F. Takeuchi, MD, Assistant Professor of Medicine, Department of Medicine, Faculty of Medicine, University of Tokyo.

Address reprint requests to Dr. M.H. Park, Department of Clinical Pathology, Seoul National University College of Medicine, 28 Yongon-dong, Chongno-gu, Seoul 110-744, Korea. E-mail: parkmhee@snu.ac.kr

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Systemic sclerosis (SSc) is a multisystem connective tissue disease of unknown etiology that is thought to have an autoimmune origin. It presents clinically and serologically as a heterogeneous disease with several highly disease-specific autoantibodies, which mainly include antibodies to centromere and DNA topoisomerase I. Clinically, SSc can be divided into 2 major subtypes, one characterized by widespread skin involvement with more severe internal organ involvement (diffuse subtype) and the other a milder disease characterized by more restricted cutaneous involvement (limited subtype). Generally, anti-topoisomerase I (anti-topo I) antibody and anti-centromere antibody (ACA) are associated with the diffuse and limited subtypes, respectively[1-5].

Recent investigations on the immunogenetic background of the disease have shown that HLA class II alleles are associated with the disease. These HLA associations have been shown to be more strongly allied to specific autoantibodies rather than to the disease itself[6-8]. Thus, SSc patients with

anti-topo I or ACA have been shown to have distinct associations with HLA-DR or DQ alleles. HLA-DRB1*1104 in Caucasians[9-11] and DRB1*1502 in Japanese[12,13] were found to be associated with anti-topo I positive SSc. The shared amino acid sequence between amino acids 67 and 71 (FLEDR: Phe, Leu, Glu, Asp, Arg) of the DR-ß chain encoded by most DRB1*11 and *08 alleles and by DRB5*0102 (which is in linkage disequilibrium with DRB1*1502) was suggested to be a candidate epitope associated with the anti-topo I response[11-13]. In some immunogenetic studies it has appeared more likely that HLA-DQB1 alleles are primarily associated with these autoantibodies. Strong associations of DQB1 alleles with anti-topo I positive SSc have been reported for DQB1*0301 in several ethnic groups, including American Caucasians and Blacks as well as Choctaw Indians[9,10,14], and for DQB1*0601 and *0301 in Japanese[12]. A tyrosine residue at amino acid position 26 or 30 (Tyr-26 or Tyr-30) and the shared amino acid sequence at positions 71–77 (TRAELDT: Thr, Arg, Ala, Glu, Leu, Asp, Thr) of the DQ-ß chain encoded by DQB1*0301 and *0601 alleles were given particular importance in anti-topo I production. Primary associations of DQB1 alleles with ACA positive SSc also have been reported. Associations of DQB1*0501 and DQB1*0301 were reported for the ACA response in Caucasians, with a polar glycine or tyrosine at position 26 (Gly-26 or Tyr-26) of DQB1 molecules[15,16].

The frequencies of different autoantibodies in patients with SSc show considerable variation among different ethnic groups[7,17-20]. Multiple factors linked to ethnicity, including genetic and environmental factors, appear to influence autoantibody status in SSc[21]. Thus, more information from different ethnic groups would give insight into the exact relationship between immunogenetic background of the disease and related autoantibody response. The association of HLA with SSc has not been studied in Asian populations, other than Japanese. We investigated the HLA associations with SSc in Korean patients according to anti-topo I, ACA, and anti-U1 ribonucleoprotein (RNP) autoantibody status, and compared these results with the associations among various ethnic groups.

MATERIALS AND METHODS

Patients and controls. We studied 74 unrelated Korean patients with SSc (65 women and 9 men, age range 20–75 yrs) who visited the Rheumatology Clinic, Seoul National University Hospital, during the period January 1996 to February 1999. For controls, we included 200 healthy Koreans. All patients met the preliminary criteria of the American College of Rheumatology for SSc[22]. Patients were classified into the 2 major clinical subtypes of limited and diffuse skin involvement. Limited subtype was defined by skin involvement of the hands, face, feet and forearms; diffuse subtype was defined by acral and truncal skin involvement[23]. In addition, patients having either diffuse or limited skin involvement and typical features of one or more of the other connective tissue diseases were further categorized as "overlap syndrome"[24]. Serum samples were obtained from all SSc patients at the time of diagnosis and tested for anti-topo I, ACA, and anti-U1 RNP. Anti-topo I was detected by double immunodiffusion using rabbit thymus extract (Immunovision, Springdale, AR, USA) as an antigen source. ACA was identified by characteristic discrete speckled nuclear staining patterns on indirect immunofluorescence stain, using a commercial HEp-2 cell substrate (Kallestad™ Quantafluor kit, Sanofi Diagnostic Pasteur, Chaska, MN, USA). Anti-U1 RNP was detected by ELISA using a commercial kit, in which 3 kinds of recombinant RNP (70K protein, A peptide and D peptide) were used as antigen sources (Mesacup-2 test RNP; Medical & Biological Laboratories Co., Nagoya, Japan).

HLA typing. HLA-DRB1, DRB5, and DQB1 genotyping was performed by DNA typing methods. HLA-DRB typing was in 2 steps. Low resolution HLA-DR typing was carried out by the polymerase chain reaction-reverse hybridization method using the Amplicor® HLA DRB Test (Roche Diagnostic System, Branchburg, NJ, USA). For high resolution HLA-DRB1 and DRB5 typing, group-specific amplifications and single strand conformation polymorphism (SSCP) analyses were performed as described[13,25] with minor modifications. For HLA-DQB1 typing, combination of PCR-restriction fragment length polymorphism (RFLP) and PCR-SSCP was performed as described[26].

Statistical analysis. Phenotype frequencies of HLA alleles in patients and controls were compared using the chi-square test or Fisher's 2 tailed exact test. Relative risk was calculated using the standard Woolf method or Haldane's modified formula. Significant p values were corrected (p_{corr}) for multiple comparisons by multiplying the total number of alleles observed at each HLA locus: for DRB1 alleles × 26, for DRB5 alleles × 3, and for DQB1 alleles × 14.

RESULTS

Clinical subtypes and autoantibody status. Among 74 patients with SSc, 34 were determined to have diffuse and 40 limited skin involvement. As well, 16 (22%) SSc patients belonged to the "overlap syndrome" showing features of other connective tissue diseases: systemic lupus erythematosus (7 cases), polymyositis (5 cases), dermatomyositis (one case), rheumatoid arthritis (2 cases), or mixed connective tissue disease (one case). Anti-topo I, ACA, and anti-U1 RNP were positive in 35 (47%), 3 (4%), and 19 (26%) patients, respectively. Among anti-U1 RNP positive patients, one and 4 patients were also positive for ACA and anti-topo I, respectively. Altogether 70% (52/74) of patients were positive for one or more of these 3 autoantibodies: 65% (22/34) of the diffuse and 75% (30/40) of the limited subtype lSSc. Anti-topo I was positive in 19 (56%) of 34 patients with diffuse subtype and in 16 (40%) of 40 patients with limited subtype. No significant association was observed between anti-topo I antibody and clinical subtypes. All 3 ACA positive patients had limited subtype, but the ACA positive rate in patients with limited SSc (3/40, 8%) was rather low. Anti-U1 RNP was positive in 5 (15%) and 14 (35%) of the patients with diffuse and limited subtypes, respectively, and in 10 (63%) of the 16 patients with overlap syndrome.

HLA-DR associations with anti-topo I antibody. Because there were only 3 patients who were positive for ACA, HLA associations were examined mainly according to the presence or absence of anti-topo I antibody. Table 1 shows the frequencies of HLA-DRB1 and DRB5 alleles in SSc patients and controls. DRB1*1502 was detected significantly more frequently in anti-topo I positive SSc patients

Table 1. HLA-DRB1 and DRB5 frequencies in patients with systemic sclerosis (SSc) and controls.

HLA-DRB1 and DRB5	Anti-topo I-Positive SSc n = 35 (%)	Anti-topo I-Negative SSc n = 39 (%)	Controls, n = 200 (%)
DRB1*0101	1 (3)	4 (10)	28 (14)
1501	9 (26)	13 (33)*	34 (17)
1502	8 (23)†	1 (3)	9 (5)
0301	1 (3)	1 (3)	7 (4)
0401	0 (0)	1 (3)	1 (1)
0403	3 (9)	3 (8)	10 (5)
0404	0 (0)	0 (0)	4 (2)
0405	5 (14)	7 (18)	32 (16)
0406	1 (3)	2 (5)	21 (11)
0407	0 (0)	0 (0)	2 (1)
0410	2 (6)	0 (0)	4 (2)
1101	4 (11)	3 (8)	13 (6)
1201	2 (6)	3 (8)	18 (9)
1202	4 (11)	2 (5)	18 (9)
1301	1 (3)	0 (0)	6 (3)
1302	3 (9)	4 (10)	31 (16)
1401	1 (3)	0 (0)	12 (6)
1402	0 (0)	0 (0)	2 (1)
1403	0 (0)	1 (3)	5 (3)
1405	2 (6)	4 (10)	15 (8)
1407	0 (0)	0 (0)	1 (2)
0701	8 (23)	3 (8)	27 (14)
0802	5 (14)	2 (5)	11 (6)
0803	3 (9)	11(28)**	30 (15)
0901	2 (6)	8 (21)	33 (17)
1001	0 (0)	4 (10)	9 (5)
DRB5*0101	9 (26)	13 (33)*	34 (17)
0102	8 (23)***	1 (3)	9 (5)

*p = 0.019 vs controls. †p = 0.0001 (p corrected = 0.003) vs controls and p = 0.009 vs anti-topo I negative SSc patients. **p = 0.045 vs controls. ***p = 0.0001 (p corrected = 0.0003) vs controls and p = 0.009 (p corrected = 0.027) vs anti-topo I negative SSc patients.

compared with controls (23% vs 5%; relative risk 6.3, p = 0.0001, p_{corr} = 0.003) as well as with anti-topo I negative SSc patients (23% vs 3%; RR 11.3, p = 0.009). In anti-topo I negative patients, DRB1*1501 (33% vs 17% in controls; p = 0.019) and *0803 (28% vs 15% in controls; p = 0.045) were somewhat increased in frequencies. HLA-DRB1*1501 and *1502 alleles were completely associated with DRB5*0101 and *0102, respectively, in both patients and controls.

It was noteworthy that DRB1*1502, which showed the strongest association with anti-topo I response, was detected in only 23% of the anti-topo I positive patients with SSc. To determine whether any other DRB1 alleles are associated with this form of the disease, we analyzed the DRB1*1502 negative patients for 5 DRB1 (*0701, *0802, *1101, *1202, *1501) alleles, and the results are shown in Table 2. These DRB1 alleles belong to either responder alleles with topo I-specific proliferative response[27] or those with shared amino acid sequences of ^{67}FLEDR^{71} [11-13]. All these 5 alleles were

Table 2. HLA-DRB1 frequencies in patients with systemic sclerosis (SSc) and controls among DRB1*1502-negative individuals.

HLA-DRB1*	Anti-topo I Positive SSc, n = 27 (%)	Anti-topo I Negative SSc, n = 38 (%)	Controls, n = 191 (%)
DRB1*0701	7 (26)	3 (8)	26 (14)
0802	5 (19)†	2 (5)	10 (5)
1101	4 (15)	3 (8)	13 (7)
1202	3 (11)	2 (5)	18 (9)
1501	8 (30)	12 (32)	34 (18)
Responder group	15 (56)	16 (42)	71 (37)
^{67}FLEDR^{71} group	17 (63)†	16 (42)	71 (37)
^{38}V-^{67}FLEDR^{71} group	8 (30)†	5 (13)	22 (12)
^{38}L-^{67}FLEDR^{71} group	11 (41)	12 (32)	50 (26)

*Responder group of Kuwana, *et al*[27]: DRB1*0701, 1101, 1501, 1502; ^{67}FLEDR^{71} group: DRB1*0802, 1101, 1202, 1501, 1502; ^{38}V-^{67}FLEDR^{71} group: DRB1*0802, 1101, 1502; ^{38}L-^{67}FLEDR^{71} group: DRB1*1202, 1501. †p = 0.011 vs controls.

somewhat increased in frequencies in anti-topo I positive patients; however, statistical significance was observed only for DRB1*0802 (19% vs 5% in controls; p = 0.011). Anti-topo I positive patients without DRB1*1502 also showed a significantly increased frequency of ^{67}FLEDR^{71} group alleles including DRB1*0802, *1101, *1202, and *1501 (63% vs 37% in controls; p = 0.011). Among these, only those alleles with a valine at amino acid position 38 (^{38}V-^{67}FLEDR^{71}) were increased including DRB1*0802 and *1101 (30% vs 12% in controls; p = 0.011). The ^{38}V-^{67}FLEDR^{71} group alleles altogether including DRB1*0802, *1101, and DRB5*0102 (exclusively associated with DRB1*1502) showed the strongest association with anti-topo I response (46% in anti-topo I positive patients vs 16% in controls, p = 0.00004, p_{corr} = 0.001; vs 15% in anti-topo I negative patients, p = 0.004). ^{38}L-^{67}FLEDR^{71} group alleles were not associated with anti-topo I response.

HLA-DQ associations with anti-topo I antibody. HLA-DQB1 frequencies in SSc patients and controls are shown in Table 3. Frequencies of DQB1 alleles were not significantly different between patients and controls, except for DQB1*0402. DQB1*0402 was increased in anti-topo I positive patients compared with controls (17% vs 7%; p = 0.033) and with anti-topo I negative patients (17% vs 3%; p = 0.047). However, the increase of DQB1*0402 in anti-topo I positive patients was attributed to DRB1*0802 in close linkage disequilibrium with DQB1*0402, and there was no significant difference in the frequency of DQB1*0402 between patients and controls among DRB1*0802 negative individuals. The frequencies of DQB1 alleles with amino acid residues (Tyr-26, Tyr-30, ^{71}TRAELDT^{77}), reported to be associated with anti-topo I response[9,10,12], were not different between anti-topo I positive patients and controls as well as anti-topo I negative patients.

Table 3. HLA-DQB1 frequencies in patients with systemic sclerosis (SSc) and controls.

HLA-DQB1	Anti-topo I Positive SSc, n = 35 (%)	Anti-topo I Negative SSc, n = 39 (%)	Controls, n = 200 (%)
DQB1*0501	3 (9)	8 (21)	37 (19)
0502	1 (3)	1 (3)	6 (3)
0503	2 (6)	3 (8)	20 (10)
0601	9 (26)	11 (28)	31 (16)
0602	9 (26)	12 (31)	33 (17)
0603	1 (3)	0 (0)	7 (4)
0604	2 (6)	4 (10)	25 (13)
0609	1 (3)	0 (0)	8 (4)
02	9 (26)	1 (3)	32 (16)
0301	9 (26)	8 (21)	53 (27)
0302	6 (17)	6 (15)	39 (20)
0303	5 (14)	12 (31)	42 (21)
0401	3 (9)	7 (18)	32 (16)
0402	6 (17)*	1 (3)	13 (7)
Amino acid residues†			
Glycine-26	14 (40)	20 (51)	98 (49)
Tyrosine-26	16 (46)	18 (46)	81 (41)
Tyrosine-30	32 (91)	37 (95)	178 (89)
^{71}TRAELDT^{77}	32 (91)	37 (95)	174 (87)

*p = 0.033 vs controls and p = 0.047 vs anti-topo I negative SSc patients. †Amino acid residues in the HLA-DQB1 ß1 domain: glycine-26 = DQB1*0501, 0502, 0503, 0401, 0402; tyrosine-26 = DQB1*0601, 0301; tyrosine-30 = DQB1*0601, 0602, 0609, 0301, 0302, 0303, 0401, 0402; ^{71}TRAELDT^{77} = DQB1*0601, 0602, 0603, 0604, 0609, 0301, 0302, 0303.

HLA-DR and DQ associations with clinical subtypes and anti-topo I status. As increased frequencies of DRB1*1501 and *0803 were observed in anti-topo I negative SSc patients compared with controls (Table 1), we further analyzed the frequencies of DRB1*1501 and *0803 and related DQB1 alleles according to clinical subtypes (Table 4). Among anti-topo I negative SSc patients, the frequency of DRB1*1501 was significantly increased in patients with limited subtype disease (50% vs 17% in controls; $p < 0.001$, $p_{corr} < 0.01$, RR 4.9) and that of DRB1*0803 was increased in patients with diffuse subtype disease (47% vs 15% in controls; $p = 0.002$, $p_{corr} < 0.05$, RR 5.0). HLA-DQB1*0602 and *0601, which are closely linked to DRB1*1501 and *0803, respectively, also showed similar increases, which remained significant after correction for multiple comparisons.

HLA-DR and DQ associations with ACA. Each of 3 SSc patients with ACA had one DRB1*1501 allele and one DQB1*0602 allele as a heterozygote, indicating the possibility of the association between ACA and DRB1*1501 or DQB1*0602 (for both, 100% vs 17% in controls; p = 0.006 and p = 0.005, respectively). These 3 ACA positive patients had DQB1 genotype of *0601/*0602 in one and *0301/*0602 in 2 patients: all 3 patients belonged to Tyr-26/Tyr-30 heterozygotes and ^{71}TRAELDT^{77} homozygotes. Although the number of subjects was too small to be conclusive, these associations are consistent with those of other studies[9,10,12]. Meanwhile, exclusion of the 3 ACA positive/anti-topo I negative patients carrying DRB1*1501 did not greatly influence the association of DRB1*1501 with anti-topo I negative, limited subtype SSc (Table 4), although the level of significance was somewhat decreased (43% vs 17% in controls; p = 0.004).

HLA-DR and DQ associations with anti-U1 RNP. Patients with anti-U1 RNP did not show associations with HLA-DR or DQ alleles or with amino acid residues of DR or DQ molecules. The HLA-DRB1*0803 and *1501 alleles that showed associations with anti-topo I negative patients of diffuse and limited subtypes, respectively (Table 4), were not related with anti-U1 RNP.

DISCUSSION

This study examined the frequencies of HLA-DRB1 and DQB1 alleles in Korean patients with SSc, in relation with anti-topo I, ACA, and anti-U1 RNP autoantibodies. Because there were so few patients with ACA (n = 3) and patients with anti-U1 RNP showed no association with HLA alleles, HLA association was mainly analyzed for anti-topo I antibody status. The results indicate that HLA-DR gene has a primary association with anti-topo I positive SSc.

Table 4. Association of HLA-DRB1 and DQB1 alleles with systemic sclerosis (SSc) according to clinical subtypes and anti-topo I autoantibody status.

HLA	Anti-topo I Positive SSc		Anti-topo I Negative SSc		
	Diffuse, n = 19 (%)	Limited, n = 16 (%)	Diffuse, n = 15 (%)	Limited, n = 24 (%)	Controls, n = 200 (%)
DRB1*1502	5 (26)*	3 (19)†	0 (0)	1 (4)	9 (5)
DRB1*1501	7 (37)†	2 (13)	1 (7)	12 (50)**	34 (17)
DRB1*0803	2 (11)	1 (6)	7 (47)***	4 (17)	30 (15)
DQB1*0601	5 (26)	4 (25)	7 (47)***	4 (17)	31 (16)
DQB1*0602	7 (37)†	2 (13)	1 (7)	11 (46)**	33 (17)

*p = 0.0002 (p corrected = 0.005) vs controls. †p < 0.05 vs controls. **p < 0.001 (p corrected < 0.01) vs controls. ***p = 0.002 (p corrected < 0.05) vs controls.

DRB1*1502 showed the strongest association with anti-topo I antibody, as had been reported in Japanese. Among anti-topo I negative patients, DRB1*0803 and DRB1*1501 were associated with the diffuse and limited subtypes of the disease, respectively, which have not been described in other studies. Changes in the frequencies of DQB1 alleles were considered to arise from linkage disequilibrium of DQB1 alleles with those DRB1 alleles primarily associated with the anti-topo I response or different disease subsets.

In our study, DRB1*1502 showed the strongest association with anti-topo I positive SSc (Table 1). This finding was consistent with 2 studies in Japanese[12,13]. On the other hand, DRB1*11, especially *1104, showed the strongest association with anti-topo I response in American and UK Caucasian patients with SSc[9-11]. DRB1*1502 and DRB1*1101/*1104 must be the most representative alleles associated with anti-topo I positive SSc in Japanese and Korean populations and Caucasians, respectively. Although DRB1*1502 showed the strongest association with anti-topo I response in Korean patients with SSc, the frequencies of DRB1*1502 were rather low in both anti-topo I positive patients and controls (23% vs 5%) compared with those in Japanese (63–75% vs 18–21%)[12,13].

We analyzed the DRB1*1502 negative patients for HLA-DR association with anti-topo I response (Table 2). DRB1*0802 was significantly associated with anti-topo I response in DRB1*1502 negative patients (19% vs 5% in controls; $p = 0.01$), and this finding is consistent with that observed in Japanese[13]. Significant increase of DRB1*0802 was also reported in SSc patients among Choctaw Indians[14]. HLA-DR ^{67}FLEDR^{71} sequence also showed a significant association with anti-topo I response in DRB1*1502 negative patients, and it is of interest that only ^{38}V-^{67}FLEDR^{71} and not ^{38}L-^{67}FLEDR^{71} was associated with anti-topo I response, as described by Takeuchi, *et al*[13]. In the present study, ^{38}V-^{67}FLEDR^{71} group alleles including DRB5*0102 (exclusively associated with DRB1*1502), DRB1*0802, and DRB1*1101 were most significantly increased in anti-topo I positive SSc patients compared with controls (46% vs 16%; $p_{corr} = 0.001$). DRB1*1104, which shows a strong association with anti-topo I response in Caucasian patients, also contains the ^{38}V-^{67}FLEDR^{71} sequence. Thus HLA-DR ^{38}V-^{67}FLEDR^{71} sequence appears to be the most plausible "shared epitope" responsible for anti-topo I response in our population as well as in Caucasians.

HLA-DQ alleles are typically in linkage disequilibrium with HLA-DR alleles, and several studies have suggested that HLA-DQ alleles represent the primary association. A primary association of HLA-DQB1 gene with anti-topo I response in SSc was reported in American Caucasians and Blacks[9,10]. HLA-DQ alleles possessing Tyr-30 amino acid residue or ^{71}TRAELDT^{77} sequence were observed in 100% of anti-topo I positive patients, and thus were considered to be most closely associated with genetic predisposition to anti-topo I response in SSc[9,10]. It was also postulated that HLA-DR and DQ genes together control the autoimmune response to topo I in Japanese with SSc[12]. DQ alleles possessing Tyr-26 amino acid residue (DQB1*0601, *0301) were considered to form the minimum requirement for anti-topo I response, and DR alleles with ^{67}FLEDR^{71} sequence to control the intensity for its response[12].

However, in our study of Koreans it is evident that HLA-DQ gene is not associated with anti-topo I response in SSc (Table 3). The frequencies of HLA-DQ alleles with Tyr-26, Tyr-30, or ^{71}TRAELDT^{77} in anti-topo I positive patients were quite similar to those in controls as well as in anti-topo I negative patients. A slight increase in the frequency of DQB1*0402 was considered to be secondary to the increased frequency of DRB1*0802, which is in linkage disequilibrium with DQB1*0402. HLA-DQB1*0601, which showed a significant association with anti-topo I response in Japanese with SSc[12], is in strong linkage disequilibrium with DRB1*1502 and *0803 in both Koreans and Japanese[28]. In Korean patients with SSc, DRB1*1502 and *0803 were increased in anti-topo I positive and negative groups, respectively, and thus the frequency of DQB1*0601 was not different between these groups (26% vs 28%) (Tables 1 and 3). DQB1*0301 frequency was also not different between these groups (26% vs 21%). Putting together the results of HLA-DR and DQ studies in Japanese[12] and Koreans with SSc, it can be concluded that HLA-DR has a primary association with anti-topo I response. In this context, it is worth noting that T cell proliferative response and antibody synthesis induced by topo I peptides in SSc patients is restricted mainly by HLA-DR[27,29].

In this study, anti-topo I negative SSc patients showed a modest increase in the frequency of DRB1*1501 and DRB1*0803 compared with controls (Table 1). Among these anti-topo I negative patients, the diffuse and limited subtypes of SSc were significantly associated with DRB1*0803 (47% vs 15% in controls; $p_{corr} < 0.05$) and *1501 (50% vs 17% in controls; $p_{corr} < 0.01$), respectively (Table 4). Similarly, anti-topo I negative diffuse and limited SSc were significantly associated with DQB1*0601 and *0602, which are in close linkage disequilibrium with DRB1*0803 and *1501, respectively. It is also notable that DQB1*0601, which had been shown to have a significant association with anti-topo I response in Japanese with SSc[12], was not associated with anti-topo I response but with the anti-topo I negative limited subtype of SSc in Koreans. To our knowledge, associations of DRB1*0803 or *1501 alleles with SSc have not been reported in any other ethnic groups, including Japanese. However, it has not been investigated thoroughly in other studies, with an approach of subdividing anti-topo I positive and negative groups into the diffuse and limited clinical subsets of the disease. These new HLA associations in Koreans with SSc were not related with ACA or anti-U1 RNP responses, and whether they are

related with autoantibody subsets not tested in this study remains to be confirmed.

Systemic sclerosis is a serologically heterogeneous disorder, and various specificities of antinuclear antibodies are identified in SSc, including anti-topo I, ACA, anti-RNA polymerase, anti-U1 RNP, anti-U3 RNP, anti-PM-Scl, and anti-Th/To antibodies[3,20,30-33]. These SSc associated autoantibodies vary in frequency among different ethnic groups[7,17–20]. We investigated anti-topo I, ACA, and anti-U1 RNP, and these 3 autoantibodies accounted for 70% of Korean patients with SSc. The frequency of anti-topo I in Koreans with SSc (47%) was higher than North American Caucasians (around 20%) and Japanese (28%)[5,7]. Anti-U1 RNP was common in our patients (26%), occurring at a high frequency (63%) among patients with overlap syndrome, and these findings are quite similar to those from Japan[20]. In contrast, the frequency of ACA in our patients (4%) was much lower compared with North American Caucasians (20–51%) or Japanese (11–16%)[5,7,13]. It was comparable to the very low frequency observed in Thai patients (2%); however, these patients were exclusively of diffuse subtype[17]. Generally, anti-topo I and ACA are known to be more frequently associated with the diffuse and limited subtypes of SSc, respectively[1-5]. As well, ACA is the most commonly occurring autoantibody among patients with limited SSc, which is consistent across Japanese, Caucasian, and Black patients[20]. But our data indicate that the frequency of ACA in Koreans with limited SSc was quite low (8%), although 75% of these patients had one or more of the 3 autoantibodies tested in this study.

Koreans are closest to Japanese in the distribution of HLA alleles and haplotypes. We show that the distribution of SSc related autoantibodies and their association with HLA class II genes in Koreans have similarities and some differences compared with Japanese. We have added further evidence that the production of SSc related autoantibodies is associated with immunogenetic backgrounds and other factors linked to ethnicity.

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