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REVIEW

The role of common protective alleles *HLA-DRB1*13* among systemic autoimmune diseases

H Furukawa[1,2], S Oka[1,2], N Tsuchiya[1], K Shimada[3,4], A Hashimoto[3], S Tohma[2] and A Kawasaki[1]

Associations between human leukocyte antigen (*HLA*) and susceptibility to systemic autoimmune diseases have been reported. The predisposing alleles are variable among ethnic groups and/or diseases. On the other hand, some *HLA* alleles are associated with resistance to systemic autoimmune diseases, including systemic sclerosis, systemic lupus erythematosus and rheumatoid arthritis. Interestingly, *DRB1*13* alleles are the protective alleles shared by multiple autoimmune diseases. *DRB1*13:01* allele is protective in European populations and *DRB1*13:02* in Japanese. Because alleles in multiple *HLA* loci are in strong linkage disequilibrium, it is difficult to determine which of the protective alleles is functionally responsible for the protective effects. Thus far, association studies suggested that *DRB1*13:02* represents at least one of the causally associated protective factors against multiple systemic autoimmune diseases in the Japanese population. The protective effect of *DRB1*13* alleles appears to overcome the predisposing effect of the susceptible alleles in heterozygous individuals of *DRB1*13* and the susceptible allele. A gene dosage effect was observed in the associations of *DRB1*13:02* with the protection from systemic autoimmune diseases; thus homozygous individuals are more effectively protected from the systemic autoimmune diseases than heterozygotes. *DRB1*13:02* also confers protection against organ-specific autoimmune diseases and some infectious diseases. Several hypotheses can be proposed for the molecular mechanisms of the protection conferred by *DRB1*13*, some of which can explain the dominant effect of DRB1*13 molecules over the susceptible alleles, but the actual protective function of DRB1*13 requires further study. Understanding of the protective mechanisms of *DRB1*13* may lead to the identification of targets for the curative treatment of systemic autoimmune diseases.

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INTRODUCTION

The term 'collagen diseases' was originally proposed for a group of systemic diseases characterized by widespread fibrinoid degeneration of collagen and includes systemic autoimmune diseases such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and systemic sclerosis (SSc).[1] Although the etiology of these systemic autoimmune diseases is still unknown, it is thought that susceptibility to systemic autoimmune diseases is associated with genetic, environmental and stochastic causes. Predisposing genetic factors for systemic autoimmune diseases include the human leukocyte antigen (*HLA*) class II alleles,[2–4] which are the strongest genetic factors in almost all systemic autoimmune diseases.

HLA class II gene cluster is encoded in the 0.9 M base region on human chromosome 6 and are composed of >30 loci.[5] The genes coding HLA class II molecules are located in this region and >3500 alleles were reported. The *HLA* alleles coded on these loci are in strong linkage disequilibrium (LD), making it highly difficult to determine the functionally relevant protective gene in this region. There are at least six loci for *HLA class II* genes; *DRA*, *DRB1* (*DRB3*, *DRB4* or *DRB5* located in some haplotypes as copy number variations), *DQA1*, *DQB1*, *DPA1* and *DPB1* encode the α and β chains of HLA-DR, -DQ and -DP molecules, respectively (Figure 1). HLA-DR, -DQ and -DP molecules were heterodimers formed by the 32 kD α and 28 kD β chains. HLA class II molecules present primarily exogenous peptides to T-cell receptors of $CD4^+$ T cells, stimulating acquired immunity.

PREDISPOSING EFFECTS OF *HLA* ON SYSTEMIC AUTOIMMUNE DISEASES

Skewed *HLA* class II allele frequencies are associated with systemic autoimmune diseases. SSc is a chronic systemic autoimmune disease that is featured by skin and internal organ fibrosis. Antinuclear antibodies are frequently detected in SSc patients. Genetic risk factors for SSc include *HLA* class II alleles as the strongest ones. *HLA-DRB1*11:04*, *DQB1*03:01* and *DQB1*26 epi* (*DQB1* alleles encoding a non-leucine residue at position 26 of the HLA-DQβ chain) are associated with SSc susceptibility in Europeans[6] and *DRB1*15:02*, *DPB1*03:01* and *DPB1*09:01* in Asians.[7–10] It was also known that *DRB1*08:04* and *DQB1*03:01* are associated with SSc in African-American and that *DRB1*11:04* and *DQB1*03:01* are associated in Hispanic populations.[6]

Anti-centromere antibodies (ACA)[11] and anti-topoisomerase I antibodies (ATA)[12] are detected in SSc patients and suggested to define subsets of SSc. It has been shown that ACA-positive SSc was associated with *DQB1*05:01* and *DQB1*26 epi* in European populations[6] but with *DRB1*01:01*, *DRB1*10:01*, *DRB1*15:02*, *DQB1*05:01*, *DPB1*03:01* and *DPB1*04:02* in Asians.[8–10,13] *DPB1*13:01* is reported to be associated with ATA-positive SSc in

[1]Molecular and Genetic Epidemiology Laboratory, Faculty of Medicine, University of Tsukuba, Tsukuba, Japan; [2]Clinical Research Center for Allergy and Rheumatology, National Hospital Organization Sagamihara National Hospital, Sagamihara, Japan; [3]Department of Rheumatology, National Hospital Organization Sagamihara National Hospital, Sagamihara, Japan and [4]Department of Rheumatic Diseases, Tokyo Metropolitan Tama Medical Center, Fuchu, Japan. Correspondence: Dr H Furukawa, Molecular and Genetic Epidemiology Laboratory, Faculty of Medicine, University of Tsukuba, 1-1-1 Tennodai, Tsukuba 305-8575, Japan.
E-mail: furukawa-tky@umin.org
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Figure 1. HLA class II gene organization of each haplotype. *DRB3* is located in the haplotype of *DRB1*03*, *DRB1*11*, *DRB1*12*, *DRB1*13* and *DRB1*14*. *DRB4* is included in the haplotype of *DRB1*04*, *DRB1*07* and *DRB1*09*. *DRB5* is found in the haplotype of *DRB1*15* and *DRB1*16*.

Europeans[6] and *DRB1*15:02*, *DRB1*16:02*, *DQB1*06:01*, *DPB1*03:01*, *DPB1*09:01* and *DPB1*13:01* in Asians.[8–10,13] Thus different *HLA* class II alleles are associated with the risk of overall SSc or subsets of SSc in different ethnic groups.

SLE is a prototypic and systemic autoimmune disease that affects multiple organs. Several different autoantibodies are detected in SLE patients. *HLA* is one of the important genetic risk factors for SLE. *DRB1*03:01* and *DRB1*15:01* are associated with SLE susceptibility in European[14,15] and *DRB1*09:01*, *DRB1*15:01* and *DRB1*15:02* in Asian populations.[16–19] It was also known that *DRB1*15:03* is associated with SLE in African-American populations[20] and that *DRB1*08:02* is associated with Hispanic populations.[21] With respect to the specific autoantibodies to ribonucleoprotein, *DPB1*05:01* was associated with SLE patients with anti-Ro/SS-A or anti-La/SS-B antibodies.[22] The association of polymorphisms of amino-acid residues 11 and 13 of DRβ molecule with SLE was reported.[23] These amino-acid residues form the HLA-DR peptide-binding groove.[24] Thus different *DRB1* alleles are associated in different ethnic groups also in the case of SLE.

RA is a chronic systemic autoimmune disease that mainly affects synovial joints, but extra-articular manifestations are often complicated. Association between RA and *HLA* has been known for 40 years.[25] RA risk is associated with some *HLA-DRB1* alleles.[26] Amino-acid sequence at positions 70–74 (QKRAA, RRRAA or QRRAA) of the HLA-DRβ chain is conserved among these alleles and was referred to as the 'shared epitope' (SE).[26] *DRB1*04:01* and *DRB1*04:05* are associated with RA in European and Asian populations, respectively.[26,27] Such difference could be explained by the different frequencies of these RA risk alleles in different ethnicities. A gene dosage effect was reported in RA but not in SLE; having two copies of SE alleles confer higher RA risk than those with one copy of SE. Although all the known genetic risk factors could explain 16% of RA risk, *HLA* alleles can explain 11%.[28,29] Anti-citrullinated peptide antibody (ACPA) is specifically detected in RA patients and is suggested to be pathogenic. SE alleles are strongly associated with ACPA-positive RA but only weakly with ACPA-negative RA.[30] The association of *HLA-DRB1* amino-acid residues with RA was also analyzed, and the important role of the polymorphisms in amino-acid positions 11 and 13 of DRβ molecule was reported.[31] The amino-acid residues of positions 11 and 13 form the HLA-DR peptide-binding groove.[24] Thus *HLA* is the most important genetic risk factor for RA, and the main predisposing alleles are different among ethnic groups. In addition, the major predisposing alleles are different among systemic autoimmune diseases.

PROTECTIVE EFFECTS OF *HLA* ON SYSTEMIC AUTOIMMUNE DISEASES

Although many studies reported susceptible associations of *HLA* class II alleles with systemic autoimmune diseases,[2–4] few attempts have been made on the protective association of *HLA*. *DRB1*07:01*, *DRB1*15:01*, *DQB1*02:02* and *DQB1*06:02* alleles were reported to be protectively associated with SSc in European populations[6] and *DRB1*01:01*, *DRB1*04:06*, *DRB1*07:01*, *DRB1*13:02*, *DRB1*14:06*, *DQB1*03:01* and *DPB1*02:01* in Asians.[9,10,13] With respect to SLE, *DRB1*13* is protective against European SLE,[32,33] and *DRB1*13:02* and **14:03* have been shown to be protective in Japanese population.[19]

In the case of RA, it had been suggested that an amino-acid sequence (DERAA) at positions 70–74,[34] isoleucine at position 67 (I67),[35] aspartic acid at position 70 (D70)[36] or a conserved amino-acid sequence at positions 71–74 (S1; ARAA or ERAA) [37,38] in the HLA-DRβ chain were shown to be protective.

Of particular interest, *DRB1*13:01* and *DRB1*13:02* are commonly present in all these protective allele groups. *DRB1*13* alleles were reported to be protectively associated with RA in European populations.[39,40] *DRB1*13:01* allele was protective against ACPA-positive RA in European populations.[41] The protective effect was attributed to *DRB1*13* rather than DERAA, D70 or I67.[41] It was recently shown that *HLA-DRB1*13* affects the onset of ACPA-positive RA but not protective against ACPA production in individuals without RA.[42] *DRB1*13* was also protective against RA in Turkish[43] and Asian populations.[44] *DRB1*13:02* is protectively associated against ACPA-positive and ACPA-negative RA in Japanese.[27,45] Thus *HLA* is one of the important resistant factors for systemic autoimmune diseases, and *DRB1*13* are the shared protective alleles against multiple diseases,

THE ROLE OF *HLA-DRB1*13:02* IN SYSTEMIC AUTOIMMUNE DISEASES

*DRB1*13:02* is carried by the extended haplotype *A*33:03-C*14:03-B*44:03-DRB1*13:02-DQB1*06:04-DPB1*04:01*, which has been reported to be positively selected in Japanese.[46] LD between *DRB1*13:02* was also observed in *HLA-G* located on the telomeric side of *HLA-A*;[47] thus LD with *DRB1*13:02* extends across almost the whole *MHC* region. Therefore, certain allele(s) on this haplotype is thought to have a causative protective for systemic autoimmune diseases. In fact, the causative allele may not necessarily be single, and multiple protective alleles of different loci may have a protective role independently.

Haplotype association analyses provide valuable information in elucidating the causatively associated alleles among a group of alleles in LD. LD between *DRB1*13:02* and *DQB1*06:04* or *DQB1*06:09* is especially strong in the Japanese population.[48] The haplotype carrier frequencies of both *DRB1*13:02-DQB1*06:04* and *DRB1*13:02-DQB1*06:09* in systemic autoimmune disease patients were obtained from secondary analyses based on the previously published data,[13,19,27] suggesting that *DRB1*13:02* rather than *DQB1* alleles has the protective role (Table 1). In addition, two-locus analysis and conditional logistic regression analysis between these alleles revealed that the primary protective effect is neither *DQB1*06:04* nor *DQB1*06:09* but *DRB1*13:02* (Tables 2 and 3). Thus the primary protective role of *DRB1*13:02* with Japanese SSc, SLE and RA was suggested.

It was known that *DRB1*13:02* and *DQA1*01:02* alleles are in strong LD in the Japanese population.[48] *DRB1*15:01* or *DRB1*16:02* alleles are also in strong LD with *DQA1*01:02*; however, neither *DRB1*15:01* nor *DRB1*16:02* conferred protective effects for systemic autoimmune diseases.[13,19,27] Thus *DQA1*01:02* is unlikely to be the functional protective allele for systemic autoimmune diseases. Similarly, *DRB1*13:02* and *DRB3*03:01* alleles are in strong LD in the Japanese population.[48] *DRB1*12:02* and *DRB3*03:01*

Table 1. *HLA* allele and haplotype carrier frequencies in SSc, SLE and RA patients and controls

	SSc (n = *459*)	*SLE* (n = *459*)	*RA* (n = *1479*)	*SSc, SLE, RA* (n = *2397*)	*Control* (n = *413*)	P	*OR*	Pc	*95% CI*
*DRB1*13:02*	32 (7.0)	30 (6.5)	107 (7.2)	169 (7.1)	57 (13.8)	1.32×10^{-5}	0.47	0.0004	(0.34–0.65)
*DQB1*06:04*	30 (6.5)	28 (6.1)	100 (6.8)	158 (6.6)	50 (12.1)	0.0002	0.51	0.0032	(0.37–0.72)
*DQB1*06:09*	3 (0.7)	1 (0.2)	4 (0.3)	8 (0.3)	6 (1.5)	0.0103	0.23	0.1538	(0.08–0.66)
**13:02-*06:04*	29 (6.3)	28 (6.1)	100 (6.8)	157 (6.5)	50 (12.1)	0.0002	0.51	0.0075	(0.36–0.71)
**13:02-*06:09*	3 (0.7)	1 (0.2)	3 (0.2)	7 (0.3)	6 (1.5)	0.0067	0.20	0.2401	(0.07–0.59)

Abbreviations: CI, confidence interval; HLA, human leukocyte antigen; OR, odds ratio; *P*c, corrected *P*-value; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SSc, systemic sclerosis. Allele and haplotype carrier frequencies are shown in parenthesis (%). Association was tested by Fisher's exact test using two-by-two contingency tables in the comparison of systemic autoimmune disease patients and controls.

alleles are also in strong LD, but *DRB1*12:02* did not show protective association against systemic autoimmune diseases.[13,19,27] Thus *DRB3*03:01* allele is unlikely to be the primary protective allele for systemic autoimmune diseases.

These haplotype analyses supported the primary protective role of *DRB1*13:02* among the *HLA* class II genes in the Japanese. However, the possibility that other gene(s) on the *DRB1*13:02*-extended haplotype, including those on the class I and class II regions, has a functional role cannot be excluded. In fact, it is possible that multiple genes on this haplotype may independently have a functional role. Such possibility will be addressed by comparison of the re-sequencing data of the *MHC* region of the *DRB1*13:02* haplotype.

In the protective associations of *DRB1*13:02* with the systemic autoimmune diseases (Table 4), a gene dosage effect was observed. Homozygosity of *DRB1*13:02* more effectively prevents the development of systemic autoimmune diseases than heterozygous *DRB1*13:02* genotypes.

*DRB1*13* has also been shown to be protective for other systemic autoimmune diseases, including anti-neutrophil cytoplasmic antibody-associated vasculitis,[49–51] mixed connective tissue disease[52] and polymyositis/dermatomyositis.[53] In addition, it was also reported that *DRB1*13:02* confers protection in organ-specific autoimmune diseases, such as psoriasis,[33,54] autoimmune hepatitis,[55] primary biliary cirrhosis,[56] Graves' disease and Hashimoto's thyroiditis.[57] *DRB1*13:02* is also a protective allele for cervical cancer caused by human papilloma virus infection,[58] severe malaria[59] and chronic hepatitis B infection.[60] In addition, *DRB1*13:02* is associated with slow disease progression in HIV infection.[61] As *DRB1*13* molecules proficiently stimulate $CD4^+$ T cells,[62,63] it appears possible that *DRB1*13:02* might be protective for putative undiscovered infectious diseases that trigger autoimmune diseases. Such a hypothesis might explain the role of *DRB1*13:02* for the protection of systemic and organ-specific autoimmune diseases.

POTENTIAL MOLECULAR MECHANISMS OF *HLA-DRB1*13*

*DRB1*13:01* is protective against ACPA-positive RA[40,41,] and SSc[64] in European populations, while *DRB1*13:02* allele was protectively associated with RA[27] and SSc[13] in Japanese populations. The only difference in the amino-acid sequence between these two alleles is at position 86 (V in *DRB1*13:01* and G in *DRB1*13:02*) of the HLA-DRβ chain. It is plausible that common protective mechanisms are present between *DRB1*13:01* and *DRB1*13:02* against systemic autoimmune diseases. In support of this hypothesis, a common peptide (TPKIQVYSRHPAENGKSN) derived from β_2-microglobulin has been shown to be presented by *DRB1*13:01* and *DRB1*13:02* molecules.[65]

When we examined the association of each amino-acid residue with systemic autoimmune diseases, the protective role was mapped to the amino-acid position 13S of the HLA-DRβ chain (Figure 2). This amino-acid residue constitutes the HLA-DR peptide-binding groove[24] and is shared between *DRB1*13:01* and *DRB1*13:02* molecules.

The protective effects of the *DRB1*13* alleles can overcome the predisposing effects of susceptible alleles in *DRB1* heterozygous RA patients.[27,41] Similar tendency was also observed in SLE[19] and SSc[13] patients heterozygous for *DRB1* in Japanese populations. To explain the dominant effects of protective *DRB1*13* alleles, it was hypothesized that resistant *DRB1*13* molecules are recognized by the T-cell receptors of autoreactive regulatory T cells with high affinity.[66]

A recent study made an attempt to explain the protection mediated by *DRB1*13* molecules for RA by the DERAA motif at positions 70–74 of the HLA-DRβ chain.[67] This motif was shared with vinculin and microbe-derived proteins. Citrullinated vinculin is one of the autoantigens of ACPA and self-reactive $CD4^+$ T cells. The motif is presented by predisposing DQ molecules and stimulates self-reactive $CD4^+$ T cells, resulting in the triggering of arthritis. However, these self-reactive $CD4^+$ T cells could not be found in *DRB1*13* possessing individuals. The motif of *DRB1*13* molecules was thought to mediate the central tolerance, explaining the protective mechanisms of *DRB1*13* molecules.

Non-inherited maternal antigen was reported to have protective roles in the pathogenesis of RA. It was observed that the resistant *DRB1* alleles with DERAA epitope at positions 70–74 of the HLA-DRβ chain, including *DRB1*13*, have protective effects on children, though these alleles were not inherited from their mothers.[68] This could be explained by the maternal micro-chimerism in the circulation of the children.

Thus several hypotheses have been proposed to explain the protective molecular mechanisms of *DRB1*13* with systemic autoimmune diseases. These hypotheses, along with other possibilities, need to be validated in future studies.

CONCLUSION

Recent studies demonstrated the protective effect of *DRB1*13* with systemic autoimmune diseases. *DRB1*13:01* is protective in European populations and *DRB1*13:02* allele in Japanese. As the ethnic difference of *HLA* allele distributions is well known, the protective effects of *HLA* alleles for systemic autoimmune diseases in other populations should be explored. Because *DRB1*13* is carried by extended haplotypes formed by *HLA* class II and class I alleles, it is quite difficult to identify which of the alleles is functionally responsible for the protective effects; however, several lines of evidence suggest that *DRB1*13* alleles themselves, at least in part, have a role, although independent effects from other genes in LD cannot be excluded. Several hypotheses have been proposed to explain the protective molecular mechanisms of *DRB1*13* molecules against systemic autoimmune diseases, some of which can explain the dominant effects of *DRB1*13* molecules. The precise understanding of the protective mechanisms of

Table 2. *HLA-DRB1* or *DQB1* allele carrier frequency in the SSc, SLE and RA patients with specific *DRB1* or *DQB1* alleles (two-locus analysis)

DRB1-DQB1	*DQB1**	*SSc, SLE, RA* (n = *2397*)		*Control* (n = *413*)		P	*OR*	Pc	*95% CI*	*DRB1**	*SSc, SLE, RA* (n = *2397*)		*Control* (n = *413*)		P	*OR*	Pc	*95% CI*
SSc, SLE, RA		*DRB1 allele positivity*		*DRB1 allele positivity*							*DQB1 allele positivity*		*DQB1 allele positivity*					
		(+)	*(−)*	*(+)*	*(−)*						*(+)*	*(−)*	*(+)*	*(−)*				
**13:02-*06:04*	(+)	156 (98.7)	2 (1.3)	50 (100.0)	0 (0.0)	1.0000	0.62	NS		(+)	156 (92.3)	13 (7.7)	50 (87.7)	7 (12.3)	0.2901	1.68	NS	
	(−)	13 (0.6)	2226 (99.4)	7 (1.9)	356 (98.1)	0.0147	0.30	0.4704	(0.12–0.75)	(−)	2 (0.1)	2226 (99.9)	0 (0.0)	356 (100.0)	1.0000	0.80	NS	
**13:02-*06:09*	(+)	8 (100.0)	0 (0.0)	6 (100.0)	0 (0.0)	1.0000	1.31	NS		(+)	8 (4.7)	161 (95.3)	6 (10.5)	51 (89.5)	0.1234	0.42	NS	
	(−)	161 (6.7)	2228 (93.3)	51 (12.5)	356 (87.5)	0.0002	0.50	0.0049	(0.36–0.70)	(−)	0 (0.0)	2228 (100.0)	0 (0.0)	356 (100.0)	1.0000	0.16	NS	

Abbreviations: CI, confidence interval; HLA, human leukocyte antigen; OR, odds ratio; NS, not significant; *Pc*, corrected *P*-value; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SSc, systemic sclerosis. Allele carrier frequencies are shown in parenthesis (%). Association was tested by Fisher's exact test using two-by-two contingency tables.

Table 3. Conditional logistic regression analysis between the protective *HLA* alleles in SSc, SLE and RA patients

SSc, SLE, RA (n = *2397*)	P	*OR (95% CI)*	$P_{adjusted}$	$OR_{adjusted}$ *(95% CI)*	$P_{adjusted}$	$OR_{adjusted}$ *(95% CI)*	$P_{adjusted}$	$OR_{adjusted}$ *(95% CI)*	$P_{adjusted}$	$OR_{adjusted}$ *(95% CI)*
Control (n = *413*)	*Unconditioned*		*Conditioned on DRB1*13:02*		*Conditioned on DQB1*06:04*		*Conditioned on DQB1*06:09*		*Conditioned on DQB1*06:04 and DQB1*06:09*	
*DRB1*13:02*	2.17×10^{-7}	0.47 (0.35–0.62)	NA	NA	0.0010	0.32 (0.16–0.63)	2.82×10^{-6}	0.49 (0.37–0.66)	0.0262	0.40 (0.18–0.90)
*DQB1*06:04*	0.0001	0.53 (0.39–0.72)	0.2278	1.57 (0.75–3.27)	NA	NA	0.0001	0.53 (0.39–0.72)	NA	NA
*DQB1*06:09*	0.0063	0.23 (0.08–0.66)	0.1370	0.43 (0.14–1.31)	0.0060	0.22 (0.08–0.65)	NA	NA	NA	NA

Abbreviations: CI, confidence interval; HLA, human leukocyte antigen; NA, not applicable; OR, odds ratio; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SSc, systemic sclerosis. *P*, OR, 95% CI, $P_{adjusted}$ and $OR_{adjusted}$ were calculated by logistic regression analysis under the additive model.

Table 4. *HLA-DRB1* genotype frequency in the SSc, SLE and RA patients and controls

	SSc, SLE, RA (n = *2397*)	*Control* (n = *413*)	P	*OR*	*95% CI*
**13:02/*13:02*	7 (0.3)	5 (1.2)	0.0173	0.22	(0.07–0.71)
**13:02*/other than **13:02*	162 (6.8)	52 (12.6)	0.0001	0.50	(0.36–0.69)
Other than **13:02*/other than **13:02*	2228 (92.9)	356 (86.2)			

Abbreviations: CI, confidence interval; HLA, human leukocyte antigen; OR, odds ratio; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SSc, systemic sclerosis. Genotype frequencies are shown in parenthesis (%). Association was tested by Fisher's exact test using two-by-two contingency tables compared with the genotype of 'other than **13:02*/other than **13:02*'.

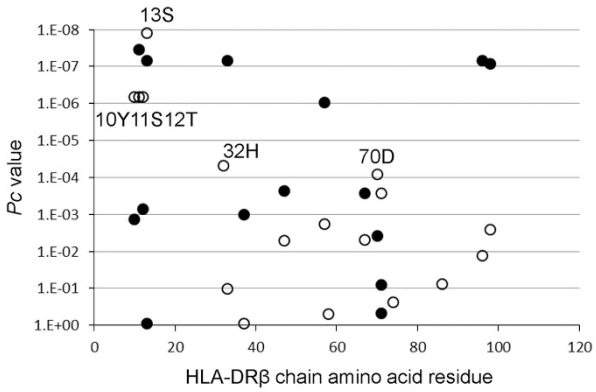

Figure 2. Associations of amino-acid residues in the DRβ chain with systemic autoimmune diseases, including SSc, SLE and RA. Corrected *P* (*Pc*) values were calculated by multiplying the *P*-value by the number of amino-acid residues tested. Associations were analyzed by Fisher's exact test using two-by-two contingency tables in the comparison of systemic autoimmune disease patients ($n = 2397$) and healthy controls ($n = 413$). Protective associations are shown by open circles and predisposing associations by filled circles.

*DRB1*13* might eventually lead to cellular, molecular or genetic targets for the permanent curative treatment of systemic autoimmune diseases.

CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: HF, NT, and ST; performed the experiments: HF, SO, and AK; analyzed the data: HF, contributed reagents/materials/analysis tools: HF, KS, AH, and ST; wrote the manuscript: HF, NT, and ST.

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