Journal of Autoimmunity 57 (2015) 53–59

Contents lists available at ScienceDirect

Journal of Autoimmunity

journal homepage: www.elsevier.com/locate/jautimm

The amino acid variation within the binding pocket 7 and 9 of HLA-DRB1 molecules are associated with primary Sjögren's syndrome

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ARTICLE INFO

Article history:
Received 19 November 2014
Accepted 27 November 2014
Available online 9 January 2015

Keywords:
Sjögren's syndrome
Autoimmunity
HLA
Peptide binding groove
Homology modeling

ABSTRACT

Primary Sjögren's syndrome (pSS) is associated with HLA-DRB1 loci, but the association of amino acid variations in the hypervariable region of the HLA-DR β1 chain with pSS is largely unknown. In this study, we aimed to identify the amino acid variations within the hypervariable region of HLA-DRB1 molecule which are associated with the susceptibility to pSS. We sequenced the 2nd exon of the HLA-DRB1 locus in 52 pSS patients and 179 controls. The HLA-DRB1*0803 is the allele that shows the strongest association with pSS in Chinese population (OR = 3.0, $P = 2.4 \times 10^{-4}$). Furthermore, amino acid variations within the binding pocket P7 and P9 are associated with the susceptibility to pSS. An interaction between two residues within P7, β47 and β67, is associated with pSS. Structural modeling studies demonstrated that the electrostatics of peptide binding pocket 9 are opposite in pSS-susceptible and -protective HLA-DRB1 alleles. In conclusion, our results suggest that structural heterogeneity of the HLA-DRB1 peptide binding pocket P7 and P9 might play a role in the pathogenesis of pSS.

1. Introduction

Primary Sjögren's syndrome (pSS) is an autoimmune disorder targeting the exocrine glands and leading to mucosal and conjunctival dryness [1]. As for most autoimmune diseases, the HLA class II loci have been convincingly demonstrated to be associated with the pSS [2,3]. The HLA class II alleles associated with pSS are different among populations. For example, HLA-DRB1*15 and HLA-DRB1*03 are associated with pSS in Caucasians [4], the HLA-DRB1*11 is associated with pSS in Jewish [5], while the HLA-DRB1*04 is associated with pSS in Japanese [6]. In addition, a marginal, but not significant association between HLA-DRB1*08 allele and pSS was reported in the Chinese population [6].

Although it is convincing that HLA class II loci are associated with pSS, the mechanism of the association is largely unknown. The HLA class II complex binds peptides via its hypervariable region (HVR) and presents the peptide to CD4 T cells. The highly polymorphic HLA class II alleles differ from each other mainly in amino acid residues within the HVR. Identification and characterization of the HLA class II susceptibility alleles and amino acid variations within the HVR region will help to understand the pathogenesis of the disease.

In this study, our aim was two folds. First, we took this opportunity to identify HLA-DRB1 allele(s) associated with pSS in Chinese population. Secondly, we extended this study by determining the effect of the amino acid variations within the HVR of HLA-DRB1 molecule on pSS.

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http://dx.doi.org/10.1016/j.jaut.2014.11.006
0896-8411/

2. Material and methods

2.1. Patients and controls

Peripheral blood samples were collected from thirty-one pSS patients in The First Affiliated Hospital of Xiamen University. All patients were diagnosed according to the standards defined by criteria of the American–European Consensus Group in 2002 [7]. The gender- and age-matched healthy controls were recruited from the Xiamen University Hospital. This study was approved by the ethics committee of Xiamen University.

2.2. DNA preparation

The genomic DNA was extracted from peripheral blood leukocytes using a TaKaRa Blood Genome DNA Extraction Kit (Takara Biotechnology, Dalian Co., Ltd., China) according to the manufacturer's recommendations. The amount of DNA was measured using an UV-spectrophotometer at A260/280 nm and the quality of DNA was checked by 0.8% agarose gel electrophoresis.

2.3. HLA-DR typing

Genotyping was performed by sequencing the 2nd exon of the HLA-DRB1 as previously described by Kotsch et al. [8]. Briefly, the HLA-DRB1 was amplified by 14 combinations of allele family specific primers located in introns. PCR products were sequenced by group-specific primers. The highly polymorphic sequence of each individual was first analyzed by Mixed Sequence Reader http://msr.cs.nthu.edu.tw/ [9] to distinguish two alleles sequence. Alignment was performed using WU-BLAST http://www.ebi.ac.uk/Tools/sss/wublast/ to define the HLA-DRB1 allele.

2.4. Meta-analysis

An exhaustive search of the Medline database was performed to identify eligible studies. The inclusion criteria were the following: the design should be a case-control study based on unrelated individuals. Studies were excluded if one of the following existed: (a) the design based on family or sibling pairs; (b) genotype frequencies or numbers not reported. The following information was collected from each study: author, year of publication, description of ethnicity in the study population, HLA typing technique, diagnosis criteria for pSS, and total number of cases.

For the control group in each study, we assessed departures from the Hardy–Weinberg equilibrium using the χ^2 test. The heterogeneity across studies was evaluated using Cochran's Q-statistics. The random effect model was used for meta-analysis when a heterogeneity was indicated by a significant Q-statistic ($P < 0.10$), otherwise the fixed effect model was used. The allele frequencies for the *DRB1* polymorphism were determined in each study using the allele-counting method. The odds ratio (OR), 95% confidence intervals (CI), and P values were estimated for each study by comparing the allele frequencies. Meta-analysis was performed to calculate the pooled OR, 95% CI and P values. Statistical analysis was performed using the Comprehensive Meta-Analysis computer program (Biosta, Englewood, NJ, USA).

2.5. Stepwise regression modeling

The stepwise ("forwards-backwards") procedure started by taking the best amino acids (lowest P value) into the regression model, and iteratively adding amino acids ("forwards") to evaluate if the goodness-of-fit (the Akaike Information Criterion (AIC) metric) improves. An amino acid already in the model can be ejected from the model ("backwards") if that improves the AIC. This procedure was performed in R (http://www.r-project.org) using the "glm" and "step" functions.

2.6. Statistical analyses

The analyses for association between pSS and the HLA-DR variants were performed using the χ^2 test. Results are expressed as mean ± SD. The statistical significance of differences between groups was calculated by either the chi-square test or Fisher's exact test. Power calculations were performed by using SPSS. A P value of <0.05 was considered significant.

2.7. 3D protein structure modeling of HLA-DR molecules

Graphical representations of structures were constructed with PyMOL 1.3 (Schrödinger, LLC). The structural models of pSS-susceptible and pSS-nonrelated HLA-DR molecules were constructed based on the Protein Data Bank (PDB) ID: 4MDI [10]. All the modeling structure was built through SWISS-MODEL server (http://swissmodel.expasy.org/) using the deep view project.

Electrostatic surface potential was calculated by the APBS PyMOL plugin [11], with externally generated PQR files from the PDB2PQR website (http://nbcr-222.ucsd.edu/pdb2pqr_1.8/) [12] and are colored from red (negative potential, −10 kT) to blue (positive potential, +10 kT).

3. Results

3.1. Characteristics of the dataset

We analyzed fifty-two pSS patients (7 males and 45 females) which were recruited from the First Affiliated Hospital of Xiamen University. The mean age of the patients at the time of the study was 44 ± 12.52 years (mean ± SD). Among the patients, 65.9% and 83.7% suffered xerophthalmia and xerostomia respectively. 66% of the patients had the elevated IgG levels in the sera, and 86.3% and 35.3% of the patients carried anti-SSA and anti-SSB autoantibodies respectively. 40.8% and 43.5% of the patients showed low concentration of complement 3 (C3) and C4 respectively. The histopathological evaluation of glandular biopsies showed a mean focus score of 1.92 ± 1.56. The disease duration was 27.91 ± 29.98 months.

3.2. HLA-DRB1 typing

The HLA-DRB1 typing was performed in 52 patients and 179 controls by sequencing the 2nd exon of the gene. The frequencies of HLA-DRB1 alleles in patients and controls are presented in the Supplementary Table 1. Three HLA-DRB1 alleles including DRB1*0803, DRB1*1401 and DRB1*1602 were associated with an increased risk of pSS (Table 1). The frequency of the HLA-DRB1*0803 allele in patients (22.1%) was significantly higher than that in controls (8.66%) ($P = 0.00028$, OR = 3.0, 95% CI: 1.66–5.41). The frequency of the HLA-DRB1*1401 was significantly higher only in the anti-SSB positive patients (9.30%) than in controls (3.63%) ($P = 0.03$, OR = 2.7, 95% CI: 1.09–6.79). The frequency of the HLA-DRB1*1602 was 8.6% in patients and 3.4% in controls ($P = 0.03$, OR = 2.7, 95% CI: 1.12–6.68). In addition, the HLA-DRB1*1201 allele was negatively associated with pSS, with the allele frequency of 1.0% and 6.7% in the patients and controls respectively ($P = 0.05$, OR = 0.14, 95% CI: 0.02–1.01), suggesting a protective role in the development of pSS.

Table 1
Association of HLA-DBR1 loci with pSS in Chinese population.

HLA-DRB1	n^a	%	OR (95% CI)	*P* value
0803				
control ($n = 358$)	31	8.66		
all patients ($n = 104$)	23	22.12	3.00 (1.66–5.41)	2.80E-04
anti-SSA+ ($n = 86$)	19	22.09	2.99 (1.59–5.61)	6.30E-04
anti-SSB+ ($n = 34$)	7	20.59	2.73 (1.10–6.79)	0.03[b]
1401				
control ($n = 358$)	13	3.63		
all patients ($n = 104$)	8	7.69	2.21 (0.89–5.49)	NS
anti-SSA+ ($n = 86$)	8	9.3	2.72 (1.09–6.79)	0.03[b]
anti-SSB+ ($n = 34$)	3	8.82	2.56 (0.69–9.50)	NS
1602				
control ($n = 358$)	12	3.35		
all patients ($n = 104$)	9	8.65	2.73 (1.12–6.68)	0.03[b]
anti-SSA+ ($n = 86$)	8	9.3	2.95 (1.17–7.48)	0.02[b]
anti-SSB+ ($n = 34$)	2	5.88	1.80 (0.38–8.40)	NS
1201				
control ($n = 358$)	24	6.7		
all patients ($n = 104$)	1	0.96	0.14(0.02–1.01)	0.05
anti-SSA+ ($n = 86$)	1	1.16	0.16(0.02–1.01)	NS
anti-SSB+ ($n = 34$)	0	0	0.07(0.004–1.08)	NS

Note:
[a] Number of chromosomes.
[b] Not significant after correction.

3.3. Meta-analysis of association between DRB1 alleles and pSS

We then performed a meta-analysis to confirm the novel association between the four HLA-DRB1 allele and pSS. Since the HLA-DRB1*1602 allele was studied only in this study, we combine all HLA-DRB1*16 alleles for the meta-analysis. Ten case-control studies passed the criteria relating to HLA-DRB1 polymorphisms and susceptibility to pSS were recruited for the meta-analysis [4,6,13–20], For the HLA-DRB1*0803 allele, 5 case-control studies were recruited for the meta-analysis, with 492 pSS patients and 1860 controls. The meta-analysis showed a strong association between the HLA-DRB1*0803 allele and pSS ($P = 2.79E-08$, OR = 2.77, 95% CI: 1.93–3.97) (Fig. 1(A)). For the HLA-DRB1*1401 allele, the meta-analysis with 252 patients and 1120 controls did not confirm the association between the allele and pSS ($P = 0.32$, OR = 1.43, 95% CI: 0.70–2.90) (Fig. 1(B)). The meta-analysis with 426 patients and 1310 control showed a weak association between the HLA-DRB1*16 allele and pSS ($P = 1.99E-03$, OR = 2.47, 95% CI: 1.39–4.37) (Fig. 1(C)). The negative association between the HLA-DRB1*1201 alleles and pSS was also confirmed by the meta-analysis with 252 patients and 1120 control studies ($P = 0.045$, OR = 0.23, 95% CI: 0.05–0.96) (Fig. 1(D)). Taken together, the meta-analysis confirmed that the HLA-DRB1*08:03 and HLA-DRB1*16 alleles were associated with an increased risk of pSS and the HLA-DRB1*12:01 allele was associated with a decreased risk of the disease.

3.4. HLA-DRB1 amino acid variations in pSS patients and controls

The sequence of the 2nd exon of the *HLA-DRB1* genes allowed us to determine the amino acid variations within the HVR region of the HLA-DRB1. Sequencing analysis of the polymorphic exon 2 of the DRB1 gene showed 27 polymorphic amino acid positions. Among them, 8 polymorphisms are biallelic and remaining 19 positions accommodate more than two amino acid variants. Significant differences in the frequencies of amino acids between pSS patients and controls were observed at 9 of the 27 polymorphic amino acids, at positions 13, 26, 31, 37, 47, 57, 70, 71 and 74, (Table 2). Of these, three amino acid positions were associated with an increased risk to pSS, including Gly^{13} ($P = 0.005$, OR = 1.91, 95% CI: 1.20–3.05), Ser^{57} ($P = 0.003$, OR = 2.18, 95% CI: 1.30–3.65) and Leu^{74} ($P = 0.0001$, OR = 2.89, 95% CI: 1.65–5.06). Two amino acids of Tyr^{26} ($P = 0.009$, OR = 0.35, 95% CI: 0.16–0.79) and Ile^{31} ($P = 0.038$, OR = 0.24, 95% CI: 0.06–1.03) were associated with a

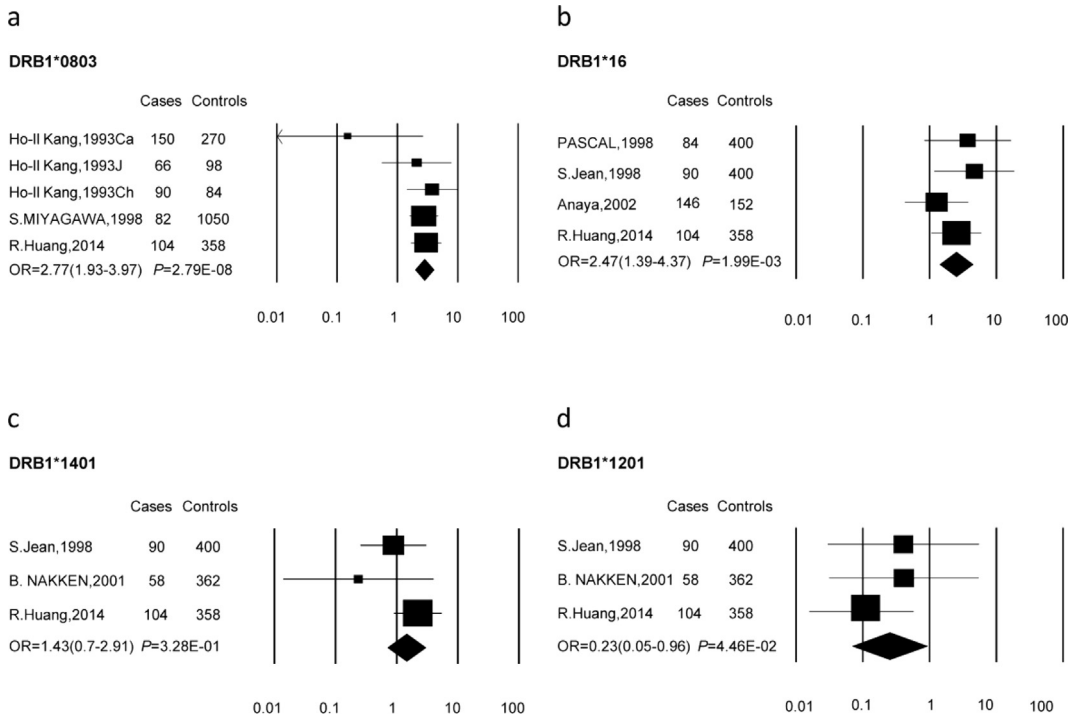

Fig. 1. Forest plot of case-control studies examining the association of pSS. Meta-analysis was performed examining the association of pSS with HLA-DRB1*0803 (a), HLA-DRB1*16 (b), HLA-DRB1*1401 (c) and HLA-DRB1*1201 (d). The odds ratio (OR), 95% confidence intervals (CI), and *P* values were estimated for each study. Meta-analysis was performed to calculate the pooled OR, 95% CI and *P* values using the Comprehensive Meta-Analysis computer program (Biosta, Englewood, NJ, USA). The size of the square represents the weight of the individual study in the meta-analysis. The diamonds represent the OR (centre line of the diamond) and 95% CI (lateral tips of the diamond) of the meta-analysis.

Table 2
HLA-DR amino acid frequencies in patients and controls.

Amino acid position	Amino acid variants	pSS (%) $n^a = 104$	Controls (%) $n^a = 358$	OR (95% CI)	*P* value
13	F	4 (3.8)	27 (7.5)	0.49 (0.17–1.43)	
	G	40 (38.5)	88 (24.6)	1.92 (1.21–3.05)	**5.37E-03**
	H	10 (9.6)	47 (13.1)	0.7 (0.34–1.45)	
	R	18 (17.3)	56 (15.6)	1.13 (0.63–2.02)	
	S	28 (26.9)	122 (34.1)	0.71 (0.44–1.16)	
	Y	4 (3.8)	18 (5)	0.76 (0.25–2.28)	
26	F	83 (79.8)	241 (67.3)	1.92 (1.13–3.25)	
	L	14 (13.5)	56 (15.6)	0.84 (0.45–1.58)	
	Y	7 (6.7)	61 (17)	0.35 (0.16–0.79)	**9.00E-03**
31	F	100 (96.1)	329 (91.9)	2.2 (0.76–6.42)	
	I	2 (1.9)	27 (7.5)	0.24 (0.06–1.03)	**0.04**
	V	2(1.9)	2(0.6)	3.49(0.49–25.08)	
37	F	17(16.3)	40(11.2)	1.55(0.84–2.87)	
	L	12(11.5)	50(14)	0.8 (0.41–1.57)	
	N	8 (7.7)	83 (23.2)	0.28 (0.13–0.59)	**4.71E-04**
	S	20 (19.2)	68 (19)	1.02 (0.58–1.77)	
	Y	47 (45.2)	117 (32.7)	1.7 (1.09–2.65)	**0.02**
47	F	37 (35.6)	180 (50.2)	0.55 (0.35–0.86)	**8.67E-03**
	Y	67 (64.4)	178 (49.7)	1.83 (1.17–2.88)	**8.67E-03**
57	A	9 (8.7)	18 (5)	1.79 (0.78–4.11)	
	D	49 (47.1)	197 (55)	0.73 (0.47–1.13)	
	S	29 (27.9)	54 (15.1)	2.18 (1.30–3.65)	**2.76E-03**
	V	17 (16.3)	89 (24.9)	0.59 (0.33–1.05)	
70	D	64 (61.5)	173 (48.3)	1.71 (1.10–2.67)	**0.02**
	Q	24 (23)	137 (38.3)	0.48 (0.29–0.80)	**4.21E-03**
	R	16 (15.4)	48 (13.4)	1.17 (0.64–2.17)	
71	A	9 (8.7)	42 (11.7)	0.71 (0.33–1.52)	
	E	1 (0.9)	11 (3.1)	0.31 (0.04–2.40)	
	K	5 (4.8)	52 (14.5)	0.3 (0.12–0.76)	**7.99E-03**
	R	89 (85.6)	253 (70.7)	2.46 (1.36–4.45)	**2.27E-03**
74	A	52 (50)	199 (55.6)	0.8 (0.52–1.24)	
	E	17 (16.3)	66 (18.4)	0.86 (0.48–1.55)	
	L	26 (25)	37 (10.3)	2.89 (1.65–5.06)	**1.25E-04**
	Q	4 (3.8)	19 (5.3)	0.71 (0.24–2.15)	
	R	5 (4.8)	37 (10.3)	0.44 (0.17–1.15)	**0.08**

pSS = Primary Sjögren's syndrome; OR = Odds Ratio; 95% CI = 95% confidence interval.*P* values in the associated amino acids were calculated with the Cochran–Armitage test. Only *P* values that were significant are shown.
[a] Number of chromosomes.

decreased risk to pSS. The other four positions at 37, 47, 70 and 71 showed discordant associations, where Tyr^{37} ($P = 0.02$, OR = 1.70, 95% CI: 1.09–2.65), Tyr^{47} ($P = 0.009$, OR = 1.83, 95% CI: 1.17–2.88), Asp^{70} ($P = 0.02$, OR = 1.70, 95% CI: 1.10–2.67) and Arg^{71} ($P = 0.002$, OR = 2.46, 95% CI:1.36–4.45) were associated with an increased risk to pSS, however, Asn^{37} ($P = 0.0004$, OR = 0.28, 95% CI: 0.13–0.59), Phe^{47} ($P = 0.009$, OR = 0.55, 95% CI: 0.35–0.86), Gln^{70} ($P = 0.004$, OR = 0.48, 95% CI: 0.29–0.80) and Lys^{71} ($P = 0.008$, OR = 0.30, 95% CI: 0.12–0.76) were associated with a decreased risk of the disease.

All of those amino acids are located in MHC-II peptide binding pockets 4, 7 and 9 (Fig. 2), Suggesting these three binding pockets are important for pSS susceptibility.

3.5. Stepwise generalized linear regression analysis to determine causative amino acids

To determine whether the association of amino acid with pSS is due to its own contribution or due to its linkage with other amino acid, we performed a stepwise regression analysis by R software [21]. Since the regression analysis included all nine positions would cause quasi-complete separation problem [22], we performed the regression analysis for two subset of amino acids. One included risk positions (13, 57, and 74) and both risk and protective positions (37, 47, 70, and 71). Another included protective positions (26, 31) and both risk and protective positions

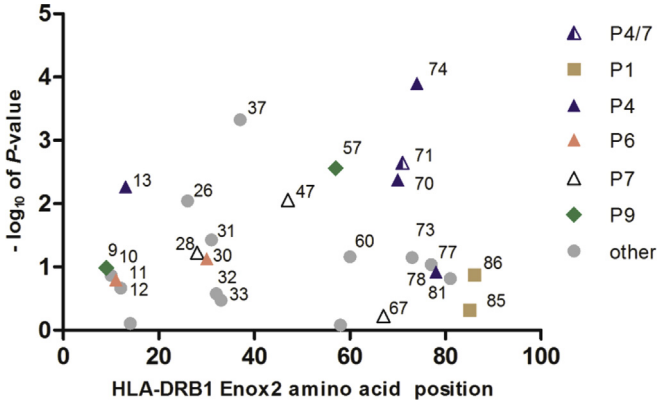

Fig. 2. Associations of amino acid variations within HLA-DRB1 hypervariable region with pSS. The numbers indicate the position of the amino acid residue. The colors denote the MHC II binding pockets of amino acids, where P1 stands for pocket 1 and et. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(37, 47, 70, and 71). The regression analysis showed an independent effect for five amino acids at position of Gly-13 (omnibus $P = 0.05$), Asn-37 (omnibus $P = 0.02$), Val-57 (omnibus $P = 0.02$), Ile-67(omnibus $P = 0.01$) and Glu-70 (omnibus $P = 0.01$). Therefore, our analysis for independent effects demonstrated that these five positions gave the main contributions to association with pSS, whereas the other five amino acid positions might associated only by linkage disequilibrium.

3.6. Effect of the interaction between two amino acids within peptide binding pocket 7

Interestingly, in contrast to HLA-DRB1*0803 and HLA-DRB1*1601, the HLA-DRB1*0802 and HLA-DRB1*1602 were not associated with pSS. HLA-DRB1*0803 differs from HLA-DRB1*0802 at the amino acid position 57 and 67, while HLA-DRB1*1602 differs from HLA-DRB1*1601 only at the position 67 (Fig. 3), indicating that the amino acid at the position 67 might contribute to the difference in association. However, individual amino acid analysis demonstrated that the amino acid at position 67 alone was not associated with the diseases.

Residue 67 of the HLA-DR β-chain is located in pocket 7, together with residues 65 and 69 of the α-chain and residues 28, 30, 47, 61, 70 of the β-chain [23]. We hypothesized that the position β67 might contribute to the diseases by interacting with other P7 residues. We then evaluated the effect of interaction between position 67 and other positions within the P7 on pSS. The interaction between position of β47 and β67 showed an effect on the risk to pSS. Position 47 is dipolymorphism with Tyr or Phe, while Ile, Leu and Phe are presented at position 67. Since both Ile and Leu are aliphatic and of similar size [24], we combined these two amino acids as one group, denoted as $(I + L)^{67}$. There are four combination of amino acids at the position 47 and 67, including Y^{47} with $(I + L)^{67}$, Y^{47} with F^{67}, F^{47} with $(I + L)^{67}$, and F^{47} with F^{67} (Table 3). Among them, combination of Y^{47} with $(I + L)^{67}$ was associated with an increased risk of pSS ($P = 0.001$, OR = 2.07, 95% CI: 1.33–3.23), while the combination of Y^{47} with F^{67} was not. The combination of F^{47} with $(I + L)^{67}$ was associated with a decrease risk of pSS ($P = 0.001$, OR = 0.39, 95% CI: 0.22–0.68), but the combination of F^{47} with F^{67} was not. Taken together, these results suggest that the interaction of the two amino acids at MHC II binding pocket 7 is associated with pSS.

Interestingly, the combination of Y^{47}–$(I + L)^{67}$ is presented all three risk HLA-DRB1 alleles including *0803, *1401 and *1602 in

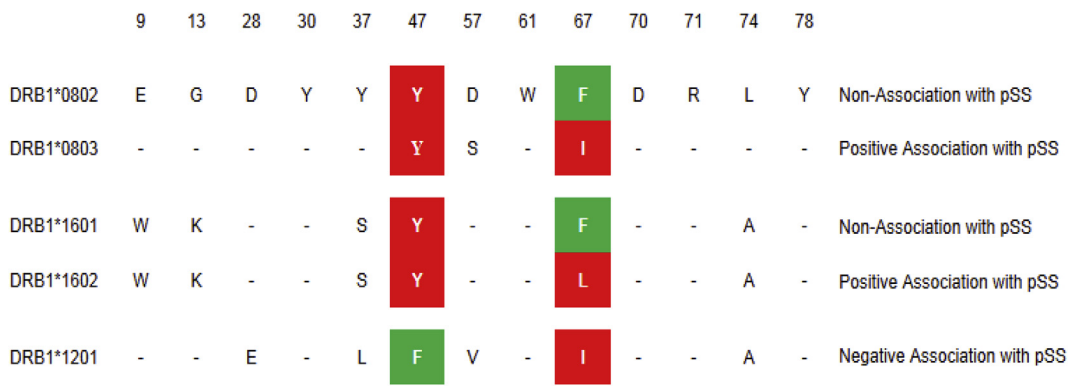

Fig. 3. Residues that differ among HLA-DRB1 alleles that are positively or negatively associated with pSS. Amino acid that identical to 0802 are shown as "-" with the exception of position β47 and β67. Risk residues at position 47 and 67 are in red color, whereas protective residues are in green.

this study, whereas the combination of $F^{47}-(I+L)^{67}$ is presented in the disease-protection associated allele HLA-DRB1*1201 (Fig. 3).

3.7. pSS susceptibility correlates with electrostatic charge differences in the P9 pockets of DRB1*0802, DRB1*0803 and DRB1*1201

Although HLA-DRB1*0803 differs from HLA-DRB1*0802 in two residues, previous studies showed the structure of them are different [25]. To compare the three-dimensional structure of pSS-non-related (HLA-DRB1*0802), pSS-susceptible (HLA-DRB1*0803) and pSS-resistant (HLA-DRB1*1201) alleles, we performed homology modeling to explore the effect of these key residues on the HLA-DR molecule (Fig. 4).

We further characterized the electrostatic potential on the surface of the HLA-DRB1 molecules. The HLA-DRB1*0802 subtype, which is not associated with pSS, has a negative electrostatics potential in pocket 9 (Fig. 4(A)). The risk allele HLA-DRB1*08:03 has a nearly neutral electrostatics potential in pocket 9 (Fig. 4(B)), while the protective allele HLA-DRB1*12:01 has a positive electrostatics potential in pocket 9 (Fig. 4(C)). These results showed that difference in the electrostatic potential of pocket 9 might confer different effects of HLA-DRB1*08:02, *08:03 and *12:01 on pSS risks.

4. Discussion

In this current study, we investigated the association between HLA-DRB1 loci and pSS in the Chinese population. Our results demonstrated that HLA-DRB1*0803 and *1602 were associated with the increased risk of pSS and the HLA-DRB1*1201 was associated with a decreased risk of disease. These associations were further confirmed using meta-analysis. In this case-control study, no association was observed between pSS and the HLA-DBR1*03, *04, *11 or *15 which were associated with pSS in other populations [5,6,14,20]. This result supports that the HLA class II alleles associated with pSS are different among populations.

HLA-DRB1*0803 is the allele shows strongest association with pSS in Chinese population, while it is not associated with the disease in Caucasian and in Jewish [5,6]. This discrepancy might due to the diversity in the frequency of the HLA-DRB1*0803 among populations, where it in south Asia is rather high (approximately 10%) while it is nearly absent in Caucasian and in Jewish [6,26].

HLA-DRB1*0803 and *0802 differ only in two amino acid positions, β47 (Phe in *0802, Tyr in *0803) in the P7 pocket and β57 (Asp in *0802, Ser in *0803) in the P9 pocket. Therefore, the two amino acid residues might play an essential role in the susceptibility to pSS.

This (Tyr/Phe 47) polymorphism is known to be important for peptide binding and presentation to T cells [27]. Unlike the very similar Phenylalanine, Tyrosine contains a reactive hydroxyl group, this difference would might affect the selectivity of the binding of pathogenic peptides to the HLA-DR pocket and thus might increase the risk for developing pSS by altering MHC-TCR peptide binding and presentation to T cells.

Our findings show an interaction between positions 47 and 67 within the P7 pocket is associated with pSS. The P7 pocket is deeper and with polarity amino acid Tyr47 in pSS-susceptible HLA-DRB1*0803 (47-Tyr and 67I or 67L) than in the pSS-resistant HLA-DRB1*1201 (47-Phe and 67-Phe) [25], suggesting that the size of pocket 7 might be important to pSS susceptibility.

Our three-dimensional modeling results shows that variation in pSS associated residues encoded by HLA-DRB1 impose distinct electrostatics characteristics on the HLA-DR peptide binding groove pocket 9. HLA-DR binding pocket electrostatics potential is critical for autoimmune disease susceptible. For example, RA susceptibility correlates with electrostatic charge differences in the P4 pockets of DRB1*0401 and DRB1*0402 [10]. It is also well established that the HLA-DQ β57 in pocket 9 has a strong influence over the shape of the peptide binding groove at this point. HLA-DQβ57 Asp gives rise to a closed groove whilst HLA-DQ β57 Ala or Ser is more 'relaxed' [28]. These differences have a major impact on the types of peptides that are presented. One example is in T1D, the amino acid residue at position 57 of the DQβ chain plays a key role in the genetic susceptibility to disease [29]. Our structure modeling results showed a distinct pocket 9 electrostatics potential in pSS-risk and

Table 3
The effect of the interaction between DRB1 β47 and β67 on pSS.

β47	β67	pSS (Frequncy)	Control (Frequncy)	OR (95% CI)	*P* value
Y	I + L	0.6	0.42	2.07 (1.33–3.23)	1.33E-03
Y	F	0.05	0.08	0.57 (0.22–1.52)	NS
F	I + L	0.16	0.34	0.39 (0.22–0.68)	9.91E-04
F	F	0.19	0.17	1.18 (0.67–2.07)	NS

Note: β47 and β67 denote the amino acid position on DRB1 β chain, which located in peptide binding pocket 7; pSS = Primary Sjögren's syndrome; OR = Odds Ratio; 95% CI = 95% confidence interval. *P* values in the associated amino acids were calculated with the χ^2 test.

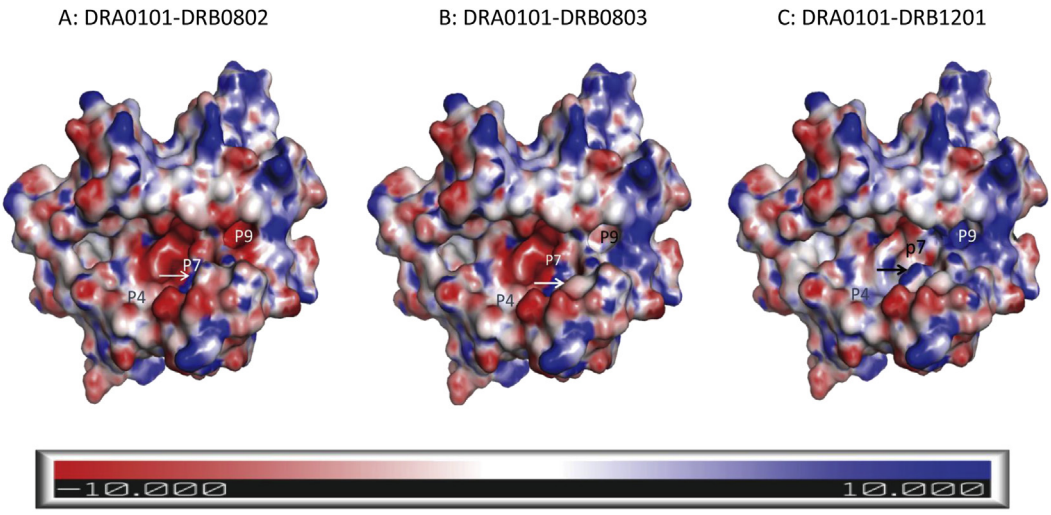

Fig. 4. Structure models of pSS-susceptible and resistant HLA/DRB1 molecules, (A) pSS unrelated HLA-DRB1*0802. (B) pSS-susceptible HLA-DRB1*0803. (C) pSS-protective HLA-DRB1*1201. Electrostatic potential maps have been calculated separately for these three molecule. A significant difference was found in P7 pocket and P9 pocket. P7 pocket in pSS-susceptible HLA-DRB1*0802 is narrow with a steeper cleft (arrow) in A, whereas wider and deeper in *0803 with a slope cleft (arrow) in B. P9 pocket has a negative electrostatics potential (red) in 0802, nearly neutral in 0803 and positive in 1201 (blue).

resistant allele, which might has an effect on autoimmune peptide presentation.

In summary, we have identified MHC class II pocket amino acid signatures that impose distinct structural and physiochemical characteristics on the HLA-DRB1 peptide binding groove and confer significant risk for pSS. The findings are highly relevant for and important to evaluate in future experimental studies of antigen presentation in pSS.

Acknowledgments

The authors would like to acknowledge and thank all the study participants. This work was supported by the National Natural Science Foundation of China (No. 81371325).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jaut.2014.11.006.

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