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Association of HLA-DQA1 and DQB1 alleles with keloids in Chinese Hans

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Summary

Background: Some studies have suggested that human HLA status might potentiate development of keloids phenotype, and exists ethnic differences. No report has been published about HLA-DQA1 and DQB1 alleles associated with keloids in Chinese Hans.
Objectives: To investigate whether HLA-DQA1 and DQB1 alleles are associated with genetic susceptibility to keloids in Chinese Hans.
Methods: Polymerase chain reaction-sequence-specific primer (PCR-SSP) method was used to analyze the distribution of HLA-DQA1 and DQB1 alleles among 192 patients with keloids and 273 healthy controls in Chinese Hans.
Results: (1) The frequencies of HLA-DQA1*0104, DQB1*0501 and DQB1*0503 (OR = 2.13, P_c = 0.0063; OR = 14.42, $P_c < 10^{-7}$ and OR = 6.09, $P_c < 10^{-7}$, respectively) were significantly higher, while the frequencies of DQA1*0501, DQB1*0201 and DQB1*0402 (OR = 0.46, P_c = 0.0099; OR = 0.24, $P_c < 10^{-4}$ and OR = 0.10, P_c = 0.0054, respectively) were lower in patients than in controls. (2) In this study significant susceptibility haplotypes to keloids were DQA1*0104—DQB1*0501 and DQA1*0104—DQB1*0503. (3) HLA-DQB1*0501 and DQB1*0503 were positively associated with all subgroups of keloid patients. However, the DQA1*0104 (OR = 2.51, P_c = 0.0009; OR = 2.22, P_c = 0.0090 and OR = 2.20, P_c = 0.0117, respectively) was only prevalent in keloid patients with single site, moderate severity and negative family history. (4) HLA-DQB1*0201 (OR = 0.27, P_c = 0.0018 and OR = 0.27, P_c = 0.0012, respectively) and DQB1*0402 (OR = 0.07, P_c = 0.0270 and OR = 0.07, P_c = 0.0306, respectively) were negatively associated with moderate severity and negative family

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history in keloids, moreover, HLA-DQB1*0201 (OR = 0.23, P_c = 0.0003) and DQA1*0501 (OR = 0.43, P_c = 0.0234) were less prevalent in patients with single site.
Conclusion: This study demonstrated the positive association of HLA-DQA1 and DQB1 alleles and haplotypes with keloids.

1. Introduction

Keloids are benign, proliferative dermal collagen growths that represent a pathological wound-healing response to skin injury in susceptible persons [1,2]. They are thick scar tissue of human skin, which have escaped the boundaries of the original wound to invade the surrounding normal skin. However, they are limited to the dermis unlike a malignant tumour [3]. Keloids were first described centuries ago in the Smith Papyrus, later in 1770 by Retz and in 1806 by Alibert, who proposed the current name [4]. Although keloids are common among the darker pigmented races, the epidemiology of keloids in general is variable. The reported incidence of keloids in the general population ranges from a high of 16% among the adults in Zaire to a low of 0.09% in England. Sizeable clinical case collections of keloids have been compiled in some countries and regions [5—8], but there are limited data on Chinese patients with keloids.

Despite the pathogenetic mechanisms that cause keloids remain unknown, some studies support the hypothesis that immunologic mechanisms play an important role in hypertrophic scarring [9,10], Castagnoli et al. [10] found a genetically determined risk factor for hypertrophic scar formation located in the HLA region. HLA-DRB-16, B14, and BW-16 have been affiliated with a predisposition to keloid formation [11].

The HLA class II locus is located in the 6p21.3 region on the short arm of chromosome 6 and encompasses approximately 700 kb. It consists of over 30 gene loci including the major class II structural genes DP, DQ and DR. While autoimmune disease correlates to specific DP, DQ or DR alleles have been documented. HLA class II molecule is critical to the development of $CD4^+$ T-lymphocyte responses through its role in antigen presentation [12]. However, no report has been published about HLA-DQA1 and DQB1 alleles associated with keloids in Chinese Hans. In order to explore the possible involvement of HLA-DQA1 and DQB1 alleles in pathogenesis of keloids, we studied the distribution of DQA1 and DQB1 alleles in patients with keloids and healthy individuals by using polymerase chain reaction-sequence-specific primer (PCR-SSP). The aim of this study was to determine whether HLA-DQA1 and DQB1 alleles are associated with genetic susceptibility to keloids in Chinese Hans.

2. Materials and methods

2.1. Patients and controls

A total of 192 unrelated Han patients (91 males and 101 females) with keloids were recruited consecutively from the outpatients at the Department of Dermatology, the First Affiliated-Hospital, Anhui Medical University. The patients ranged in age from 2 to 79 years with a mean age of 31.54 years. The healthy controls were comprised of 273 disease-free unrelated individuals, age-, sex- and ethnicity-matched with the patients from the same areas. All subjects gave their informed written consent before participation.

Patients were categorized as follows:

(1) Single site and multiple site: according to the article published by Bayat et al. [6], 'single site' keloid refers to a scar or a number of scars found in only one anatomical location or site. 'Multiple site'

Table 1a Summary of most relevant clinical data of the studied Chinese population with keloids

	Number of cases	Mean ± S.D. (years)
Gender (male/female)	91/101	
Age at diagnosis (years)		31.54 ± 13.33
Patients' characteristics		
Single/multiple site groups	132/60	
Mild/moderate/severe	38/137/17	
Positive/negative family history	57/135	

S.D.: standard deviation.

refers to scars found in a multiple number of anatomical locations as opposed to multiple scars found in the same anatomical site. In this study, the patients were divided into 'single site group' and 'multiple site group' based on this guideline. (2) Severity of keloids: clinical features of keloids included color, contour, texture, pruritus and pain. Each of these clinical features was given a score of between 0 and 3, increasing values indicating increasing keloids severity. The score ranged from 0 to 15 and was classified as <6 (mild), 6—10 (moderate) and >10 (severe) [13]. (3) Family history (positive: a patient's first and/or second-degree relatives had keloid; negative: otherwise).

Table 1a showed a summary of most relevant clinical data of the studied Chinese population with keloids in this study.

2.2. DNA preparation

Venous blood for HLA typing was collected in ethylenediamine tetraacetic acid (EDTA) anticoagulated tubes. Genomic DNA was extracted from these blood specimens by an improved salting-out method [14], and then dissolved in sterile double-distilled water for usage.

2.3. Amplification primers and PCR conditions

The amplifying primers were described by Olerup et al. [15]. Control primers giving rise to a 796-bp fragment from the third intron of HLA-DRB1 were included in all PCR reactions [16]. The conditions and parameters of amplifying reactions were also referred to the previous description. PCR reaction mixtures (10 μl) consisted of 50 ng genomic DNA, PCR buffer [50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris—HCl (pH 8.5), and 0.01% (v/v) Tween 20], 0.2 mM each of dNTP, 0.30 μM of the allele and group-specific DQA1 and DQB1 primers, 0.06 μM of the control primers and 0.5 U of Taq polymerase (MBI Fermentas). Preliminary denaturation was performed at 94 °C for 5 min, followed immediately

Table 1b Distribution of HLA-DQA1 and HLA-DQB1 in patients with keloids and controls

HLA alleles	Controls (n = 273) n (AF%)	Keloid patients (n = 192) n (AF%)	OR (95% CI)	P	P_c
DQA1*0101	50 (9.16)	19 (4.95)	0.49 (0.27—0.89)	0.0172	0.1548
DQA1*0102	40 (7.33)	42 (10.94)	1.63 (0.98—2.71)	0.0589	0.5301
DQA1*0103	55 (10.07)	44 (11.46)	1.18 (0.73—1.89)	0.5462	4.9158
DQA1*0104	52 (9.52)	64 (16.67)	2.13 (1.36—3.33)	0.0007	0.0063
DQA1*0201	49 (8.97)	27 (7.03)	0.75 (0.43—1.28)	0.3229	2.9061
DQA1*0301	104 (19.05)	76 (19.79)	1.06 (0.72—1.58)	0.8199	7.3791
DQA1*0302	81 (14.84)	49 (12.76)	0.81 (0.52—1.26)	0.3806	3.4254
DQA1*0401	0 (0.00)	0 (0.00)	—	—	—
DQA1*0501	83 (15.20)	32 (8.33)	0.46 (0.28—0.74)	0.0011	0.0099
DQA1*0601	31 (5.68)	25 (6.51)	1.17 (0.64—2.12)	0.6902	6.2118
DQB1*0201	72 (13.19)	15 (3.91)	0.24 (0.13—0.44)	$<10^{-6}$	$<10^{-4}$
DQB1*0301	49 (8.97)	32 (8.33)	0.91 (0.54—1.53)	0.8144	14.6592
DQB1*0302	13 (2.38)	15 (3.91)	1.69 (0.74—3.89)	0.2446	4.4028
DQB1*0303	79 (14.47)	52 (13.54)	0.91 (0.59—1.41)	0.7392	13.3056
DQB1*0304	0 (0.00)	0 (0.00)	—	—	—
DQB1*0401	14 (2.56)	10 (2.60)	1.02 (0.41—2.50)	0.8616	15.5088
DQB1*0402	26 (4.76)	2 (0.52)	0.10 (0.02—0.44)	0.0003	0.0054
DQB1*0501	10 (1.83)	68 (17.71)	14.42 (6.91—30.93)	$<10^{-7}$	$<10^{-7}$
DQB1*0502	12 (2.20)	6 (1.56)	0.70 (0.23—2.06)	0.6490	11.6820
DQB1*0503	12 (2.20)	42 (10.94)	6.09 (2.99—12.64)	$<10^{-7}$	$<10^{-7}$
DQB1*0504	10 (1.83)	5 (1.30)	0.70 (0.21—2.28)	0.7116	12.8088
DQB1*0601	44 (8.06)	24 (6.25)	0.74 (0.42—1.31)	0.3403	6.1254
DQB1*0602	129 (23.63)	65 (16.93)	0.57 (0.38—0.85)	0.0053	0.0954
DQB1*0603	13 (2.38)	4 (1.04)	0.43 (0.12—1.43)	0.2061	3.7098
DQB1*0604	6 (1.10)	8 (2.08)	1.93 (0.60—6.40)	0.3433	6.1794
DQB1*0605	14 (2.56)	16 (4.17)	1.68 (0.76—3.75)	0.2327	4.1886
DQB1*0606	18 (3.30)	6 (1.56)	0.46 (0.16—1.25)	0.1466	2.6388
DQB1*0607	2 (0.37)	2 (0.52)	1.43 (0.14—14.27)	1.0000	18.0000
DQB1*0608	18 (3.30)	5 (1.30)	0.38 (0.12—1.11)	0.0825	1.4850

AF: allelic frequencies; n: number of individuals; OR: odds ratio; CI: confidence interval; P: stand P-value; P_c: corrected P-value.

by 30 cycles of 30 s of denaturation at 94 °C, 30 s of primer annealing at 55—65 °C and 40 s of primer extending at 72 °C in each cycle, followed by a single round of final extension at 72 °C for 10 min. PCR products were electrophoresed in 2.0% agarose gel containing 0.5 μg/ml ethidium bromide. Gels were run for 40 min at 5 v/cm in 0.5× TBE buffer (89 mM Tris base, 89 mM boric acid and 2 mM EDTA, pH 8.0). Gels were visualized using Gel Documentation and Analysis (Advanced American Biotechnology, Fullerton, CA, USA) after electrophoresis.

2.4. Statistical analysis

The allelic frequencies were calculated according to the formula: AF = positive frequency/2. Statistical analysis was carried out through the Epi Info (Version 6.0) package (Center for Disease Control, Atlanta, GA, USA). The significance of the distribution of the allelic frequencies between patients and controls was assessed by χ^2-test with Yate's correction, and Fisher's two-tailed exact test was used when given by Epi Info. Odds ratio (OR) was also calculated. When multiple comparisons are made, significant associations may arise by chance. To avoid this error, *P*-values were corrected (P_c) for multiple testing using Bonferroni's method; namely, P_c was corrected by multiplying the number of alleles observed. The level of $P_c < 0.05$ was accepted as statistically significant. Haplotype analysis was performed on the basis of the known association of HLA-DQA1 and DQB1 alleles in this study, and the results were confirmed by PHASE calculation software [17].

3. Results

3.1. HLA frequency

The frequencies for each allele of HLA-DQA1 and DQB1 loci identified by PCR-SSP in the 192 keloids patients and 273 healthy controls are shown in Table 1b. Some alleles of these loci are not represented in the tested subjects and this might be found only rarely (if at all) in the Chinese Han population. From Table 1b, the frequencies of HLA-DQA1*0104 (OR = 2.13, $P_c = 0.0063$), DQB1*0501 (OR = 14.42, $P_c < 10^{-7}$) and DQB1*0503 (OR = 6.09, $P_c < 10^{-7}$) were significantly increased in keloids patients compared with controls, while the frequencies of HLA-DQA1*0501 (OR = 0.46, $P_c = 0.0099$), DQB1*0201 (OR = 0.24, $P_c < 10^{-4}$) and DQB1*0402 (OR = 0.10, $P_c = 0.0054$) were found to be highly decreased in these patients.

3.2. Haplotype association

Significant linkage disequilibrium between DQA1*0104 and DQB1*0501 ($P < 0.0001$, OR = 12.25) or DQB1*0503 ($P = 0.0023$, OR = 6.54) were found by the analysis of two-locus haplotypes in the HLA-DQA1 and DQB1 alleles. No extended haplotype was found to be significantly related to keloids (Table 2).

3.3. HLA-different site group

We compared the distribution of HLA-DQA1 and DQB1 alleles between different site group and controls. We found the frequencies of HLA-DQB1*0501 and DQB1*0503 were obviously increased not only in single site group patients (OR = 14.07, $P_c < 10^{-7}$ and OR = 5.33, $P_c < 10^{-4}$ separately) but also in multiple site group (OR = 15.23, $P_c < 10^{-7}$ and OR = 7.91, $P_c < 10^{-5}$ separately). The significant decreased frequencies of HLA-DQA1*0501 (OR = 0.43, $P_c = 0.0234$), DQB1*0201 (OR = 0.23, $P_c = 0.0003$) and DQB1*0402 (OR = 0.15, $P = 0.0056$) in single site group were observed. Furthermore, we also found that DQB1*0402 (OR = 0.15, $P_c = 0.1008$) did not reach the level of significance after correcting for multiple testing. HLA-DQA1*0104 (OR = 2.51, $P_c = 0.0009$) was more prevalent only in single site group patients (as shown in Table 3).

3.4. HLA-severity of keloids

We analyzed the distribution of HLA-DQA1 and DQB1 alleles in the different severity of patients versus controls. The frequencies of DQB1*0501 (OR = 10.71, $P_c < 10^{-4}$; OR = 14.64, $P_c < 10^{-7}$ and OR = 23.38, $P_c < 10^{-4}$, respectively) and DQB1*0503 (OR = 4.08, $P = 0.0138$; OR = 5.84, $P_c < 10^{-5}$ and OR = 15.23, $P_c < 10^{-3}$, respectively) were obviously increased in different severity group. DQA1*0104 (OR = 2.22, $P_c = 0.0090$) was only prevalent in keloid

Table 2 HLA-DQA1 and DQB1 haplotypes associated with keloids in Chinese Hans

Haplotypes	Patients (n = 192)[a]	Controls (n = 273)[a]	OR	95% CI	*P*-value
DQA1*0104—DQB1*0501	23 (5.99)	3 (0.55)	12.25	3.43—52.04	0.0000
DQA1*0104—DQB1*0503	13 (3.39)	3 (0.55)	6.54	1.71—29.30	0.0023

[a]Values are expressed as 2*n* (%). *n*: number of individuals; OR: odds ratio; CI: confidence interval; *P*: stand *P*-value.

Table 3 The distribution of HLA-DQA1 and HLA-DQB1 in patients with single site group and multiple site group and controls

HLA alleles	Controls (n = 273) n (AF%)	Patients with keloid							
		Single site group (n = 132)				Multiple site group (n = 60)			
		n (AF%)	OR (95% CI)	P	P_c	n (AF%)	OR (95% CI)	P	P_c
DQA1*0101	50 (9.16)	15 (5.68)	0.57 (0.29—1.10)	0.1006	0.9054	4 (3.33)	0.32 (0.09—0.97)	0.0431	0.3879
DQA1*0102	40 (7.33)	30 (11.36)	1.71 (0.98—3.00)	0.0609	0.5481	12 (10.00)	1.46 (0.67—3.13)	0.4027	3.6243
DQA1*0103	55 (10.07)	30 (11.36)	1.17 (0.68—1.98)	0.6401	5.7609	14 (11.67)	1.21 (0.59—2.46)	0.7073	6.3657
DQA1*0104	52 (9.52)	49 (18.56)	2.51 (1.54—4.10)	0.0001	0.0009	15 (12.50)	1.42 (0.70—2.86)	0.3879	3.4911
DQA1*0201	49 (8.97)	17 (6.44)	0.68 (0.36—1.27)	0.2496	2.2464	10 (8.33)	0.91 (0.40—2.03)	0.9611	8.6499
DQA1*0301	104 (19.05)	54 (20.45)	1.13 (0.72—1.76)	0.6632	5.9688	22 (18.33)	0.94 (0.51—1.74)	0.9525	8.5725
DQA1*0302	81 (14.84)	35 (13.26)	0.86 (0.52—1.40)	0.5884	5.2956	14 (11.67)	0.72 (0.36—1.44)	0.4086	3.6774
DQA1*0401	0 (0.00)	0 (0)	—	—	—	0 (0)	—	—	—
DQA1*0501	83 (15.20)	21 (7.95)	0.43 (0.25—0.76)	0.0026	0.0234	11 (9.17)	0.51 (0.24—1.08)	0.0850	0.7650
DQA1*0601	31 (5.68)	12 (4.55)	0.78 (0.36—1.65)	0.6022	5.4198	13 (10.83)	2.16 (0.99—4.67)	0.0542	0.4878
DQB1*0201	72 (13.19)	10 (3.79)	0.23 (0.11—0.48)	$<10^{-4}$	0.0003	5 (4.17)	0.25 (0.09—0.69)	0.0046	0.0828
DQB1*0301	49 (8.97)	21 (7.95)	0.86 (0.48—1.56)	0.7124	12.8232	11 (9.17)	1.03 (0.47—2.22)	0.9082	16.3476
DQB1*0302	13 (2.38)	8 (3.03)	1.29 (0.48—3.44)	0.7540	13.5720	7 (5.83)	2.64 (0.90—7.53)	0.0649	1.1682
DQB1*0303	79 (14.47)	40 (15.15)	1.07 (0.66—1.72)	0.8679	15.6222	12 (10.00)	0.61 (0.29—1.27)	0.2125	3.8250
DQB1*0304	0 (0.00)	0 (0)	—	—	—	0 (0)	—	—	—
DQB1*0401	14 (2.56)	6 (2.27)	0.88 (0.29—2.53)	0.9928	17.8704	4 (3.33)	1.32 (0.35—4.52)	0.5439	9.7902
DQB1*0402	26 (4.76)	2 (0.76)	0.15 (0.02—0.65)	0.0056	0.1008	0 (0.00)	0.00 (0.00—0.81)	0.0068	0.1224
DQB1*0501	10 (1.83)	46 (17.42)	14.07 (6.51—31.16)	$<10^{-7}$	$<10^{-7}$	22 (18.33)	15.23 (6.28—37.67)	$<10^{-7}$	$<10^{-7}$
DQB1*0502	12 (2.20)	5 (1.89)	0.86 (0.26—2.69)	0.9828	17.6904	1 (0.83)	0.37 (0.02—2.81)	0.4766	8.5788
DQB1*0503	12 (2.20)	26 (9.85)	5.33 (2.47—11.68)	$<10^{-5}$	$<10^{-4}$	16 (13.33)	7.91 (3.27—19.28)	$<10^{-6}$	$<10^{-5}$
DQB1*0504	10 (1.83)	5 (1.89)	1.04 (0.30—3.38)	1.0000	18.0000	0 (0)	0.00 (0.00—2.38)	0.2185	3.9330
DQB1*0601	44 (8.06)	18 (6.82)	0.82 (0.43—1.54)	0.6152	11.0736	6 (5.00)	0.58 (0.21—1.51)	0.3166	5.6988
DQB1*0602	129 (23.63)	49 (18.56)	0.66 (0.42—1.03)	0.0690	1.2420	16 (13.33)	0.41 (0.21—0.78)	0.0056	0.1008
DQB1*0603	13 (2.38)	2 (0.76)	0.31 (0.05—1.46)	0.1589	2.8602	2 (1.67)	0.69 (0.10—3.34)	1.0000	18.0000
DQB1*0604	6 (1.10)	7 (2.65)	2.49 (0.73—8.56)	0.1305	2.3490	1 (0.83)	0.75 (0.09—6.37)	1.0000	18.0000
DQB1*0605	14 (2.56)	9 (3.41)	1.35 (0.52—3.44)	0.6457	11.6226	7 (5.83)	2.44 (0.85—6.87)	0.0757	1.3626
DQB1*0606	18 (3.30)	5 (1.89)	0.56 (0.18—1.64)	0.3605	6.4890	1 (0.83)	0.24 (0.01—1.76)	0.2166	3.8988
DQB1*0607	2 (0.37)	1 (0.38)	1.03 (0.09—11.30)	1.0000	18.0000	1 (0.83)	2.30 (0.21—25.64)	0.4501	8.1018
DQB1*0608	18 (3.30)	3 (1.14)	0.33 (0.08—1.21)	0.1098	1.9764	2 (1.67)	0.49 (0.08—2.28)	0.5476	9.8568

AF: allelic frequencies; n: number of individuals; OR: odds ratio; CI: confidence interval; P: stand P-value; P_c: corrected P-value.

Table 4 Distribution of HLA-DQA1 and HLA-DQB1 in different severity of keloids and controls

HLA alleles	Controls (n = 273) n (%)	Mild (n = 38)				Moderate (n = 137)				Severe (n = 17)			
		n (AF%)	OR (95% CI)	P	P_c	n (AF%)	OR (95% CI)	P	P_c	n (AF%)	OR (95% CI)	P	P_c
DQA1*0101	50 (9.16)	3 (3.95)	0.38 (0.09–1.37)	0.1706	1.5354	13 (4.74)	0.47 (0.23–0.93)	0.0284	0.2556	3 (8.82)	0.96 (0.21–3.74)	1.0000	9.0000
DQA1*0102	40 (7.33)	11 (14.47)	2.37 (1.01–5.48)	0.0459	0.4131	31 (11.31)	1.70 (0.98–2.96)	0.0608	0.5472	0 (0)	0.00 (0.00–1.80)	0.1420	1.2780
DQA1*0103	55 (10.07)	9 (11.84)	1.23 (0.51–2.91)	0.7709	6.9381	31 (11.31)	1.16 (0.68–1.96)	0.6502	5.8518	4 (11.76)	1.22 (0.32–4.23)	0.7570	6.8130
DQA1*0104	52 (9.52)	11 (14.47)	1.73 (0.75–3.93)	0.2274	2.0466	47 (17.15)	2.22 (1.36–3.63)	0.0010	0.0090	6 (17.65)	2.32 (0.72–7.19)	0.1191	1.0719
DQA1*0201	49 (8.97)	3 (3.95)	0.39 (0.09–1.40)	0.1855	1.6695	22 (8.03)	0.87 (0.49–1.57)	0.7348	6.6132	2 (5.88)	0.61 (0.09–2.92)	0.7458	6.7122
DQA1*0301	104 (19.05)	15 (19.74)	1.06 (0.50–2.23)	0.9886	8.8974	52 (18.98)	0.99 (0.64–1.55)	0.9359	8.4231	9 (26.47)	1.83 (0.62–5.40)	0.3363	3.0267
DQA1*0302	81 (14.84)	13 (17.11)	1.23 (0.56–2.66)	0.7021	6.3189	32 (11.68)	0.72 (0.44–1.19)	0.2179	1.9611	4 (11.76)	0.73 (0.19–2.50)	0.7852	7.0668
DQA1*0401	0 (0.00)	–	–	–	–		–	–	–		–	–	–
DQA1*0501	83 (15.20)	6 (7.89)	0.43 (0.15–1.13)	0.0938	0.8442	24 (8.76)	0.49 (0.28–0.83)	0.0073	0.0657	2 (5.88)	0.31 (0.05–1.44)	0.1667	1.5003
DQA1*0601	31 (5.68)	2 (2.63)	0.43 (0.07–1.98)	0.3980	3.5820	19 (6.93)	1.26 (0.65–2.41)	0.5663	5.0967	4 (11.76)	2.40 (0.62–8.62)	0.1343	1.2087
DQB1*0201	72 (13.19)	2 (2.63)	0.16 (0.03–0.68)	0.0078	0.1404	12 (4.38)	0.27 (0.13–0.53)	0.0001	0.0018	1 (2.94)	0.17 (0.01–1.28)	0.0810	1.4580
DQB1*0301	49 (8.97)	7 (9.21)	1.03 (0.39–2.64)	0.8774	15.7932	21 (7.66)	0.83 (0.46–1.49)	0.5989	10.7802	4 (11.76)	1.41 (0.37–4.91)	0.5251	9.4518
DQB1*0302	13 (2.38)	2 (2.63)	1.11 (0.00–5.51)	0.7032	12.6576	9 (3.28)	1.41 (0.54–3.63)	0.5935	10.6830	1 (2.94)	1.25 (0.15–10.20)	0.5793	10.4274
DQB1*0303	79 (14.47)	9 (11.84)	0.76 (0.32–1.78)	0.6302	11.3436	38 (13.87)	0.94 (0.58–1.52)	0.8903	16.0254	5 (14.71)	1.02 (0.30–3.27)	1.0000	18.0000
DQB1*0304	0 (0.00)	–	–	–	–		–	–	–		–	–	–
DQB1*0401	14 (2.56)	4 (5.26)	2.18 (0.57–7.66)	0.2532	4.5576	6 (2.19)	0.85 (0.28–2.43)	0.9292	16.7256	0 (0)	0.00 (0.00–6.08)	1.0000	18.0000
DQB1*0402	26 (4.76)	0 (0)	0.00 (0.00–1.29)	0.0554	0.9972	1 (0.36)	0.07 (0.00–0.49)	0.0015	0.0270	1 (2.94)	0.59 (0.03–4.56)	1.0000	18.0000
DQB1*0501	10 (1.83)	11 (14.47)	10.71 (3.80–30.46)	$<10^{-5}$	$<10^{-4}$	49 (17.88)	14.64 (6.81–32.29)	$<10^{-7}$	$<10^{-7}$	8 (23.53)	23.38 (6.53–85.76)	$<10^{-6}$	$<10^{-4}$
DQB1*0502	12 (2.20)	1 (1.32)	0.59 (0.03–4.57)	1.0000	18.0000	5 (1.82)	0.82 (0.25–2.59)	0.9245	16.6410	0 (0)	0.00 (0.00–7.25)	1.0000	18.0000
DQB1*0503	12 (2.20)	6 (7.89)	4.08 (1.26–12.79)	0.0138	0.2484	29 (10.58)	5.84 (2.74–12.63)	$<10^{-6}$	$<10^{-5}$	7 (20.59)	15.23 (4.31–54.05)	$<10^{-4}$	$<10^{-3}$
DQB1*0504	10 (1.83)	1 (1.32)	0.71 (0.09–5.71)	1.0000	18.0000	4 (1.46)	0.79 (0.20–2.81)	0.7817	14.0706	0 (0)	0.00 (0.00–8.94)	1.0000	18.0000
DQB1*0601	44 (8.06)	5 (6.58)	0.79 (0.25–2.27)	0.8169	14.7042	18 (6.57)	0.79 (0.42–1.47)	0.5170	9.3060	1 (2.94)	0.33 (0.02–2.43)	0.4867	8.7606
DQB1*0602	129 (23.63)	17 (22.37)	0.90 (0.43–1.88)	0.9063	16.3134	43 (15.69)	0.51 (0.32–0.80)	0.0030	0.0540	5 (14.71)	0.47 (0.14–1.47)	0.2377	4.2786
DQB1*0603	13 (2.38)	1 (1.32)	0.54 (0.03–4.16)	1.0000	18.0000	3 (1.09)	0.45 (0.10–1.72)	0.3182	5.7276	0 (0)	0.00 (0.00–6.62)	1.0000	18.0000
DQB1*0604	6 (1.10)	2 (2.63)	2.47 (0.33–14.33)	0.2541	4.5738	6 (2.19)	2.04 (0.57–7.29)	0.2269	4.0860	0 (0)	0.00 (0.00–16.27)	1.0000	18.0000
DQB1*0605	14 (2.56)	2 (2.63)	1.03 (0.00–5.04)	1.0000	18.0000	14 (5.11)	2.11 (0.91–4.85)	0.0854	1.5372	0 (0)	0.00 (0.00–6.08)	1.0000	18.0000
DQB1*0606	18 (3.30)	0 (0)	0.00 (0.00–1.96)	0.1432	2.5776	6 (2.19)	0.65 (0.22–1.79)	0.4980	8.9640	0 (0)	0.00 (0.00–4.57)	0.6106	10.9908
DQB1*0607	2 (0.37)	0 (0)	0.00 (0.00–30.10)	1.0000	18.0000	2 (0.73)	2.01 (0.20–20.14)	0.6040	10.8720	0 (0)	0.00 (0.00–70.42)	1.0000	18.0000
DQB1*0608	18 (3.30)	1 (1.32)	0.38 (0.02–2.85)	0.4873	8.7714	4 (1.46)	0.43 (0.12–1.37)	0.1853	3.3354	0 (0)	0.00 (0.00–4.57)	0.6106	10.9908

AF: allelic frequencies; n: number of individuals; OR: odds ratio; CI: confidence interval; P: stand P-value; P_c: corrected P-value.

Table 5 Distribution of HLA-DQA1 and HLA-DQB1 in familial and non-familial keloids patients and controls

Allele	Controls (n = 273) n (AF%)	Patients with keloid							
		With family history (n = 57)				Without family history (n = 135)			
		n (AF%)	OR (95% CI)	P	P_c	n (AF%)	OR (95% CI)	P	P_c
DQA1*0101	50 (9.16)	2 (1.75)	0.16 (0.03—0.71)	0.0096	0.0864	17 (6.30)	0.64 (0.34—1.21)	0.1848	1.6632
DQA1*0102	40 (7.33)	17 (14.91)	2.48 (1.21—5.02)	0.0104	0.0936	25 (9.26)	1.32 (0.74—2.37)	0.3896	3.5064
DQA1*0103	55 (10.07)	13 (11.40)	1.17 (0.56—2.43)	0.7859	7.0731	31 (11.48)	1.18 (0.70—2.00)	0.5980	5.3820
DQA1*0104	52 (9.52)	18 (15.79)	1.96 (0.99—3.87)	0.0540	0.4860	46 (17.04)	2.20 (1.34—3.60)	0.0013	0.0117
DQA1*0201	49 (8.97)	9 (7.89)	0.86 (0.36—1.96)	0.8428	7.5852	18 (6.67)	0.70 (0.38—1.31)	0.2974	2.6766
DQA1*0301	104 (19.05)	19 (16.67)	0.81 (0.43—1.54)	0.5991	5.3919	57 (21.11)	1.19 (0.76—1.85)	0.4871	4.3839
DQA1*0302	81 (14.84)	18 (15.79)	1.09 (0.56—2.11)	0.8989	8.0901	31 (11.48)	0.71 (0.43—1.17)	0.1900	1.7100
DQA1*0401	0 (0.00)	—	—	—	—		—	—	—
DQA1*0501	83 (15.20)	8 (7.02)	0.37 (0.16—0.86)	0.0187	0.1683	24 (8.89)	0.49 (0.29—0.85)	0.0091	0.0819
DQA1*0601	31 (5.68)	8 (7.02)	1.27 (0.51—3.12)	0.7305	6.5745	17 (6.30)	1.12 (0.57—2.20)	0.8402	7.5618
DQB1*0201	72 (13.19)	3 (2.63)	0.16 (0.04—0.54)	0.0010	0.0180	12 (4.44)	0.27 (0.13—0.54)	$<10^{-4}$	0.0012
DQB1*0301	49 (8.97)	11 (9.65)	1.09 (0.49—2.37)	0.9589	17.2602	21 (7.78)	0.84 (0.46—1.52)	0.6428	11.5704
DQB1*0302	13 (2.38)	2 (1.75)	0.73 (0.11—3.53)	1.0000	18.0000	13 (4.81)	2.13 (0.90—5.06)	0.0932	1.6776
DQB1*0303	79 (14.47)	16 (14.04)	0.96 (0.48—1.88)	0.9767	17.5806	36 (13.33)	0.89 (0.55—1.45)	0.7167	12.9006
DQB1*0304	0 (0.00)	—	—	—	—		—	—	—
DQB1*0401	14 (2.56)	2 (1.75)	0.67 (0.10—3.24)	1.0000	18.0000	8 (2.96)	1.17 (0.43—3.06)	0.9182	16.5276
DQB1*0402	26 (4.76)	1 (0.88)	0.17 (0.01—1.21)	0.0608	1.0944	1 (0.37)	0.07 (0.00—0.50)	0.0017	0.0306
DQB1*0501	10 (1.83)	16 (14.04)	10.26 (4.06—26.35)	$<10^{-7}$	$<10^{-5}$	52 (19.26)	16.48 (7.67—36.28)	$<10^{-7}$	$<10^{-7}$
DQB1*0502	12 (2.20)	1 (0.88)	0.39 (0.02—2.97)	0.7058	12.7044	5 (1.85)	0.84 (0.25—2.63)	0.9475	17.0550
DQB1*0503	12 (2.20)	12 (10.53)	5.80 (2.27—14.87)	0.0001	0.0018	30 (11.11)	6.21 (2.93—13.41)	$<10^{-7}$	$<10^{-7}$
DQB1*0504	10 (1.83)	1 (0.88)	0.47 (0.02—3.68)	0.6969	12.5442	4 (1.48)	0.80 (0.21—2.85)	1.0000	18.0000
DQB1*0601	44 (8.06)	11 (9.65)	1.24 (0.56—2.72)	0.6960	12.5280	13 (4.81)	0.55 (0.27—1.11)	0.1038	1.8684
DQB1*0602	129 (23.63)	21 (18.42)	0.65 (0.35—1.22)	0.1972	3.5496	44 (16.30)	0.54 (0.34—0.85)	0.0067	0.1206
DQB1*0603	13 (2.38)	1 (0.88)	0.36 (0.02—2.70)	0.4782	8.6076	3 (1.11)	0.45 (0.10—1.74)	0.3308	5.9544
DQB1*0604	6 (1.10)	4 (3.51)	3.36 (0.76—14.05)	0.0748	1.3464	4 (1.48)	1.36 (0.32—5.55)	0.7360	13.2480
DQB1*0605	14 (2.56)	5 (4.39)	1.78 (0.53—5.60)	0.3431	6.1758	11 (4.07)	1.64 (0.67—3.97)	0.3284	5.9112
DQB1*0606	18 (3.30)	1 (0.88)	0.25 (0.01—1.85)	0.2162	3.8916	5 (1.85)	0.54 (0.17—1.60)	0.3357	6.0426
DQB1*0607	2 (0.37)	2 (1.75)	4.93 (0.48—50.20)	0.1394	2.5092	0 (0)	0.00 (0.00—8.26)	1.0000	18.0000
DQB1*0608	18 (3.30)	2 (1.75)	0.52 (0.08—2.41)	0.5458	9.8244	3 (1.11)	0.32 (0.07—1.18)	0.1006	1.8108

AF: allelic frequencies; n: number of individuals; OR odds ratio; CI confidence interval; P: stand P-value; P_c: corrected P-value.

Table 6 The positive results of HLA-DQA1 and DQB1 alleles in all keloids patients and different groups

Alleles	Keloid	Different site group		Severity			Family history	
		Single site	Multiple site	Mild	Moderate	Severe	F−	F+
HLA-DQA1*0104	↑	↑			↑		↑	
HLA-DQA1*0501	↓	↓			↓(−)		↓(−)	↓(−)
HLA-DQB1*0201	↓	↓	↓(−)	↓(−)	↓		↓	↓
HLA-DQB1*0402	↓	↓(−)	↓(−)		↓		↓	
HLA-DQB1*0501	↑	↑	↑	↑	↑	↑	↑	↑
HLA-DQB1*0503	↑	↑	↑	↑(−)	↑	↑	↑	↑

Single site keloid: refers to a scar or a number of scars found in only one anatomical location or site, multiple site keloid: refers to scars found in a multiple number of anatomical locations as opposed to multiple scars found in the same anatomical site. The score <6 was classified as mild, the score ≤ 10 was classified as moderate, the score >10 was classified as severe; F+: a positive family history, F−: a negative family history. Comparing with controls, ↑: the frequency of allele was obviously increased, ↓: the frequency of allele was obviously decreased, ↑(−): increased but not significant after correction, ↓(−): decreased but not significant after correction.

patients with moderate severity group, whereas the frequencies of DQA1*0501 (OR = 0.49, $P = 0.0073$), DQB1*0201 (OR = 0.27, $P_c = 0.0018$) and DQB1*0402 (OR = 0.07, $P_c = 0.0270$) were decreased in moderate group, however, DQA1*0501 (OR = 0.49, $P_c = 0.0657$) was not significant after Bonferroni correction. There was no significant difference in other alleles and other group (Table 4).

3.5. HLA-family history of keloids

In patients with a positive family history, the frequencies of HLA-DQB1*0501 (OR = 10.26, $P_c < 10^{-5}$) and DQB1*0503 (OR = 5.80, $P_c = 0.0018$) were increased. The frequencies of DQA1*0501 (OR = 0.37, $P = 0.0187$) and DQB1*0201 (OR = 0.16, $P_c = 0.0180$) were decreased but DQA1*0501 (OR = 0.37, $P_c = 0.1683$) allele was not significant after correction. In patients with negative family history, the frequencies of DQA1*0104 (OR = 2.20, $P_c = 0.0117$), DQB1*0501 (OR = 16.48, $P_c < 10^{-7}$) and DQB1*0503 (OR = 6.21, $P_c < 10^{-7}$) were increased, whereas the frequencies of DQ A1*0501 (OR = 0.49, $P = 0.0091$), DQB1*0201 (OR = 0.27, $P_c = 0.0012$) and DQB1*0402 (OR = 0.07, $P_c = 0.0306$) were decreased, but DQA1*0501 (OR = 0.49, $P_c = 0.0819$) was not significant after Bonferroni correction (Table 5).

Table 6 showed a summary of all positive results in this study.

4. Discussion

To our knowledge, this is the first study to comprehensively investigate the association of HLA-DQA1 and DQB1 alleles with keloids in Chinese Hans by using the PCR-SSP method, while most of the previous studies focused on therapeutic approaches. These results showed that some HLA-DQA1 and DQB1 alleles are associated with keloids.

In this study, the frequencies of HLA-DQA1*0104, DQB1*0501 and DQB1*0503 were significantly higher, while the frequencies of DQA1*0501, DQB1*0201 and DQB1*0402 were lower in keloids patients than in controls. The mechanism of these associations is still not clear. HLA-DQ molecules are unique among class II antigens in that most of the variable amino acid residues are located on the α-helical part of the antigen-binding site [18]. Thus, it is speculated that HLA-DQA1*0104, DQB1*0501 and DQB1*0503 molecules could bind and present antigens more efficiently. Upon activation, the lesional lymphocytes release several cytokines, locally and transiently, that interact with specific receptors in response to different stimulation. Central to the immune hypothesis of keloids is that some of the T-cell lymphokines act on keratinocytes, fibroblasts and other cell types to induce changes characteristic of these scars. The presence and close proximity of activated T lymphocytes and antigen-presenting cells of various phenotypes in both the epidermis and dermis of hypertrophic tissues provides strong circumstantial evidence of a local immune response. However, the manner in which T cells achieve and maintain their activated state in hypertrophic tissues is not yet known, and both antigen-dependent and independent mechanisms may contribute [19]. Some previous studies have shown that different amounts of immune cells were observed in relation to keloids and these findings support the hypothesis that cell-mediated, major histocompatibility complex (MHC)-class II-restricted immune responses play an important role in the development of keloids [20]. On the contrary, the negative association of HLA-DQA1*0501, DQB1*0201 and DQB1*0402 alleles with keloids may be due to they incapability to bind and present antigens effectively. However, these hypotheses still need to be confirmed by further investigations. In other studies, in 1977 Laurentaci and Dioguardi [21] found HLA-B14 and BW16 antigens associated with keloids.

Later, Rossi and Bozzi [22] found that HLA-DR5 and DQw3 antigens associated with keloids too. However, in this study, these associations have not been convinced, which may be caused by the following reasons: (1) Serological typing of HLA cannot identify many HLA alleles that can be detected at the genomic level. The drawback of serological typing often leads to discrepancies in HLA association with a given disease. (2) The number of subjects involved in those previous studies was not large enough to evaluate statistical significance. (3) HLA allele frequencies vary between ethnic groups, so significant associations may only be detected in people of a particular ethnicity. Even in the same ethnicity, the frequency of the HLA type may be different among different geographical regions. Thus, a multicohort study is needed to examine such ethnically or regionally varied relations in the future.

In the analysis of linkage equilibrium, certain alleles occurred together more frequently than expected by chance. In this study, we found two positive haplotypes, including DQA1*0104—DQB1*0501, DQA1*0104—DQB1*0503. We thought that these two haplotypes may be risk factors to develop keloids in Chinese Hans.

We compared the distribution of HLA-DQA1 and DQB1 alleles among single site group, multiple site group patients and controls. However, up to the present, no previous studies have investigated the association of HLA and single site group, multiple site group patients. In this study, the frequency of DQA1*0104 was increased only in single site group patients, whereas DQB1*0501 and DQB1*0503 were increased not only in single site group but also in multiple site group. No locus was merely related to multiple site group. Too little is known to explain the biological mechanisms for this association. We also analyzed whether there was an association of HLA-DQA1 and DQB1 alleles with severity of keloids. There was no report about association between HLA and severity of keloids in existing data, we first performed this analysis and found the frequency of DQA1*0104 was obviously increased in moderate group, so we thought that this allele may be promoting factors in moderate group patients.

We also performed an analysis about HLA and family history, in this study, when the 192 patients with keloids were classified into two groups with a positive family history and negative family history, the frequencies of HLA-DQB1*0501 and DQB1*0503 alleles were obviously increased not only in positive family history group but also in negative family history group, whereas HLA-DQA1*0104 allele was more prevalent only in negative family history patients group.

In summary, this is the first report to elucidate HLA-DQA1 and DQB1 alleles association with keloids patients in Chinese Hans. Different ethnic populations and different geographical regions may have different allele frequencies. The findings of the research identified HLA-DQA1*0104, DQB1*0501 and DQB1*0503 as susceptibility alleles and HLA-DQA1*0501, DQB1*0201 and DQB1*0402 as protective alleles for the study population. Moreover, we found specific HLA alleles associated with single site, moderate severity, and negative family history. The findings of the research provide important information for researching the association of HLA with keloids further.

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