RESEARCH ARTICLE

HLA-A, HLA-B, HLA-DRB1 Polymorphisms and Risk of Cervical Squamous Epithelial Cell Carcinoma: A Population Study in China

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Abstract

Cervical cancer is the second most common cancer in women. HLA class I and II alleles polymorphisms have been shown to be associated with cervical cancer risk, but results have varied among different populations. In this study, the HLA-A, -B, and –DRB1 alleles among 100 southern Chinese women with cervical squamous cell carcinoma (SCC) were compared to 254 controls. Our results showed that B*51:01:02 allele frequency was significantly higher in patients with SCC than in healthy controls ($P = 3.17x\ 10^{-5}$, $P_c = 0.005$, OR = 26.7). Statistical analysis also revealed a significantly decreased frequency of B*51:01:01 ($P = 7.01x\ 10^{-4}$, $P_c = 0.03$, OR = 0.12) in patients with SCC when compared with healthy controls. These results indicate that HLA-B*51:01:02 may confer susceptibility to SCC and HLA-B*51:01:01 may contribute to resistance to the development of SCC in Chinese women. None of the HLA-A-B or HLA-A-B-DRB1 haplotypes were significantly different in cases and controls after multiple testing corrections, indicating the individual allele associations to be independent of the identified haplotypes. These results support the hypothesis that some HLA-B alleles could be involved with susceptibility for developing SCC.

Keywords: Cervical squamous cell carcinoma - human leukocyte antigens - polymorphism - susceptibility

Asian Pac J Cancer Prev, **14 (7)**, 4427-4433

Introduction

Cervical cancer is the second most common cancer in women (Armstrong, 2010). Worldwide, in 2010, cervical cancer killed about 200,000 women, of whom 46,000 were aged 15-49 years in developing countries (Forouzanfar et al., 2011). Squamous cell carcinoma (SCC) is the most common type of cervical cancer (Howlader et al., 2013) and it develops from the cells that line the inner part of the cervix. Infection with human papillomavirus (HPV) is a necessary cause of cervical cancer. However, only a small fraction of affected population progresses into cervical cancer, suggesting that other cofactors are needed for the pathogenesis. Human leukocyte antigens (HLA) play important roles in presenting foreign antigens to T lymphocytes, which mediates the host cellular immune response to HPV infection. Genetic regions encoding HLA is highly polymorphic, resulting in various products with different efficiency in presenting antigens. Therefore, HLA polymorphisms may lead to different responses to HPV infection and further contribute to the progression to cervical cancer (Breitburd et al., 1996; Zehbe et al., 2005).

Previous association studies on HLA polymorphisms and susceptibility to cervical cancer have obtained disparate results in different populations. Most studies were restricted to HLA class II alleles. For example, DRB1*0405 and DRB1*0407 were reported to confer a higher risk for cervical cancer in Hispanic populations (Apple et al., 1994) whereas DRB1*0401 was a risk factor in British populations (Cuzick et al., 2000; Odunsi et al., 1996). In Brazilian Women, DRB1*15 and DRB1*1503 were positively associated with cervical cancer (Maciag et al., 2000). In the Chinese populations, DRB1*13, DRB1*3 (17) were reported to be associated with an increased risk of cervical cancer(Zhao et al., 2013). With respect to HLA class I alleles, a decreased risk for invasive cervical cancer were observed in Chinese patients with A*0207/0215N or A*2402(Chan et al., 2005) and B*15 (Chan et al., 2006). Meanwhile, B*7 and B*15 showed constantly significant association with Cervical cancer in Caucasians (Castro et al., 2007). Currently, data on the association between both HLA class I and II polymorphisms and the development of SCC among Chinese women are still limited. Moreover, no investigations on HLA class I and class II haplotype frequencies in Chinese SCC patients have been carried out.

To investigate the association pattern between HLA

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Table 1. HLA-B Polymorphisms Among Cervical Squamous Cell Carcinoma (SCC) Patients and Controls

HLA-B	Allele	Case		Control		*P*	OR	95% CI
		Num	Frequency (%)	Num	Frequency (%)			
*07		4	2	12	2.36	1	0.84	0.27-2.65
	*07:02:01	1	0.5	10	1.97	0.307	0.25	0.03-1.97
	*07:05:01	3	1.5	2	0.39	0.143	3.85	0.64-23.23
*08	*08:01	1	0.5	0	0	-	-	-
*13		20	10	45	8.86	0.774	1.14	0.66-1.99
	*13:01:01	14	7	30	5.91	0.736	1.2	0.62-2.31
	*13:02:01	6	3	15	2.95	1	1.02	0.39-2.66
*14	*14:02:01	0	0	1	0.2	-	-	-
*15		26	13	71	13.98	0.86	0.92	0.57-1.49
	*15:01:01	2	1	6	1.18	1	0.85	0.17-4.22
	*15:02:01	14	7	40	7.87	0.834	0.88	0.47-1.66
	*15:05:01	0	0	2	0.39	-	-	-
	*15:07	0	0	1	0.2	-	-	-
	*15:11:01	1	0.5	3	0.59	-	-	-
	*15:12:01	2	1	3	0.59	0.625	1.7	0.28-10.25
	*15:13	1	0.5	0	0	-	-	-
	*15:18:01	2	1	8	1.57	0.734	0.63	0.13-3
	*15:25:01	2	1	4	0.79	0.678	1.27	0.23-7
	*15:27:01	1	0.5	2	0.39	-	-	-
	*15:32	0	0	1	0.2	-	-	-
	*15:35	1	0.5	0	0	-	-	-
	*15:58	0	0	1	0.2	-	-	-
*18	*18:02	2	1	0	0	0.081	-	-
*27		2	1	6	1.18	1	0.85	0.17-4.22
	*27:04:01	1	0.5	5	0.98	-	-	-
	*27:05:01	1	0.5	0	0	-	-	-
	*27:07:01	0	0	1	0.2	-	-	-
*35		7	3.5	16	3.15	1	1.12	0.45-2.75
	*35:01:01	6	3	12	2.36	0.835	1.28	0.47-3.45
	*35:03	1	0.5	0	0	-	-	-
	*35:03:01	0	0	3	0.59	-	-	-
	*35:05:01	0	0	1	0.2	-	-	-
*37	*37:01:01	3	1.5	8	1.57	1	0.95	0.25-3.62
*38	*38:02:01	7	3.5	22	4.33	0.786	0.8	0.34-1.91
*39		1	0.5	9	1.77	0.298	0.28	0.04-2.21
	*39:01:01	1	0.5	8	1.57	0.457	0.31	0.04-2.53
	*39:05:01	0	0	1	0.2	-	-	-
*40		36	18	75	14.76	0.428	1.27	0.82-1.96
	*40:01:01	31	15.5	62	12.2	0.37	1.32	0.83-2.1
	*40:02:01	3	1.5	4	0.79	0.412	1.92	0.43-8.65
	*40:06:01	2	1	9	1.77	0.737	0.56	0.12-2.62
***44**		**0**	**0**	**17**	**3.35**	**0.005**	**0**	**-**
	*44:02:01	0	0	5	0.98	-	-	-
	*44:03:01	0	0	5	0.98	-	-	-
	*44:03:02	0	0	7	1.38	0.2	0	-
*45	*45:01	0	0	1	0.2	-	-	-
*46	*46:01:01	33	16.5	67	13.19	0.388	1.3	0.83-2.05
*48		1	0.5	13	2.56	0.128	0.19	0.02-1.47
	*48:01:01	0	0	9	1.77	0.069	0	-
	*48:03:01	1	0.5	4	0.79	-	-	-
*50	*50:01:01	0	0	2	0.39	-	-	-
*51		15	7.5	42	8.27	0.874	0.9	0.49-1.66
	51:01:01	***2***	***1***	***38***	***7.48***	***7.01E-04[a]***	***0.12***	***0.03-0.52***
	51:01:02	***10***	***5***	***1***	***0.2***	***3.17E-05[b]***	***26.68***	***3.39-209.87***
	*51:02:01	2	1	1	0.2	0.196	5.12	0.46-56.8
	*51:02:02	0	0	1	0.2	-	-	-
	*51:06	1	0.5	0	0	-	-	-
	*51:07	0	0	1	0.2	-	-	-
*52	*52:01:01	2	1	13	2.56	0.256	0.38	0.09-1.72
*54		4	2	10	1.97	1	1.02	0.32-3.28
	*54:01:01	3	1.5	10	1.97	1	0.76	0.21-2.78
	*54:06	1	0.5	0	0	-	-	-
*55	*55:02:01	8	4	12	2.36	0.37	1.72	0.69-4.28
***56**		**6**	**3**	**4**	**0.79**	**0.037**	**3.9**	**1.09-13.96**
	***56:01:01**	**4**	**2**	**1**	**0.2**	**0.025**	**10.35**	**1.15-93.15**
	*56:03	2	1	3	0.59	0.625	1.7	0.28-10.25
*57	*57:01:01	4	2	3	0.59	0.107	3.44	0.76-15.49
*58	*58:01:01	16	8	53	10.43	0.45	0.75	0.42-1.34
*59	*59:01:01	0	0	1	0.2	-	-	-
*67	*67:01:01	2	1	4	0.79	0.678	1.27	0.23-7
*81	*81:01	0	0	1	0.2	-	-	-

-, Values are not calculated because of limited number of individuals in cases or controls; Num, Number of individuals; Alleles with significant difference as determined by chi-square test or Fisher exact test are showed in bold. Alleles with significant difference after correction for multiple comparisons are showed in bold italic; [a]$P_c = 0.03$; [b]$P_c = 0.005$

Table 2. HLA-B Polymorphisms Among Cervical Squamous Cell Carcinoma (SCC) Patients and Controls

DRB1	Allele	Case		Control		*P*	OR	CI
		Num	Frequency (%)	Num	Frequency (%)			
*01		1	0.5	7	1.38	0.453	0.36	0.04-2.94
	*01:01:01	1	0.5	6	1.18	0.68	0.42	0.05-3.51
	*01:02:01	0	0	1	0.2	1	-	-
*03	*03:01:01	14	7	40	7.87	0.834	0.88	0.47-1.66
*04		15	7.5	43	8.46	0.813	0.88	0.48-1.62
	*04:01:01	0	0	3	0.59	0.563	-	-
	*04:02:01	0	0	1	0.2	1	-	-
	*04:03:01	5	2.5	11	2.17	1	1.16	0.4-3.38
	*04:04:01	2	1	1	0.2	0.196	5.12	0.46-56.8
	*04:05:01	5	2.5	19	3.74	0.572	0.66	0.24-1.79
	*04:06:01	3	1.5	6	1.18	0.718	1.27	0.32-5.14
	*04:10	0	0	1	0.2	1	-	-
	*04:10:01	0	0	1	0.2	1	-	-
*07	*07:01:01	7	3.5	33	6.5	0.195	0.52	0.23-1.2
***08**		**18**	**9**	**21**	**4.13**	**0.027**	**2.29**	**1.19-4.4**
	***08:01:01**	**3**	**1.5**	**0**	**0**	**0.023**	**-**	**-**
	*08:02:01	0	0	1	0.2	1	-	-
	*08:03:02	15	7.5	19	3.74	0.072	2.09	1.04-4.19
	*08:09	0	0	1	0.2	1	-	-
*09		29	14.5	79	15.55	0.852	0.92	0.58-1.46
	*09:01:01	0	0	1	0.2	1	-	-
	*09:01:02	29	14.5	78	15.35	0.896	0.93	0.59-1.48
*10	*10:01:01	6	3	11	2.17	0.716	1.4	0.51-3.83
*11		8	4	30	5.91	0.437	0.66	0.3-1.47
	*11:01:01	7	3.5	28	5.51	0.385	0.62	0.27-1.45
	*11:04:01	0	0	1	0.2	1	-	-
	*11:06:01	1	0.5	1	0.2	0.486	-	-
*12		28	14	67	13.19	0.899	1.07	0.67-1.72
	*12:01:01	3	1.5	14	2.76	0.422	0.54	0.15-1.89
	*12:02:01	25	12.5	52	10.24	0.519	1.25	0.75-2.08
	*12:20	0	0	1	0.2	1	-	-
*13		9	4.5	22	4.33	1	1.04	0.47-2.3
	*13:01:01	3	1.5	5	0.98	0.694	1.53	0.36-6.47
	***13:02:01**	**0**	**0**	**11**	**2.17**	**0.04**	**0**	**-**
	*13:12:01	6	3	6	1.18	0.183	2.59	0.82-8.12
*14		16	8	42	8.27	1	0.96	0.53-1.76
	*14:01:01	2	1	15	2.95	0.175	0.33	0.08-1.47
	*14:03:01	0	0	4	0.79	0.581	-	-
	*14:04	0	0	3	0.59	0.563	-	-
	*14:05:01	3	1.5	11	2.17	0.767	0.69	0.19-2.49
	*14:07:01	0	0	1	0.2	1	-	-
	*14:10	0	0	1	0.2	1	-	-
	***14:54**	**11**	**5.5**	**7**	**1.38**	**0.006**	**4.17**	**1.59-10.9**
*15		34	17	81	15.94	0.858	1.08	0.7-1.67
	*15:01:01	22	11	64	12.6	0.694	0.86	0.51-1.43
	*15:01:02	0	0	1	0.2	1	-	-
	*15:02:01	12	6	16	3.15	0.144	1.96	0.91-4.23
*16		15	7.5	32	6.3	0.71	1.21	0.64-2.28
	*16:02:01	15	7.5	31	6.1	0.641	1.25	0.66-2.36
	*16:19	0	0	1	0.2	1	-	-

-, Values are not calculated because of limited number of individuals in cases or controls; Num, Number of individuals; Alleles with significant difference as determined by chi-square test or Fisher exact test are showed in bold

class I and II genes with SCC in Chinese women, we analyzed polymorphisms of HLA-A, -B, and -DRB1 genes in a case-control study of 100 SCC patients and 254 healthy control subjects. The diversity found in this population allowed us to investigate SCC susceptibility for a wide range of HLA alleles and haplotypes.

Materials and Methods

Subjects

This study was approved by institutional review board of the third affiliated hospital of Guangzhou Medical College. Signed informed-consent documents were obtained from all participants before they entered the study. Briefly, 254 healthy controls and 100 patients with pathologically proven cervical squamous epithelial cell carcinoma and HPV infection were included. Cases included women with cervical cancer admitted at the third affiliated hospital of Guangzhou Medical College for surgery or women who had histopathological confirmation of cervical squamous epithelial cell carcinoma between 2008 and 2009. Subjects with any prior treatment for cervical disease or hysterectomy were excluded. Cell specimens from controls were collected by using cytobrushes, and tumor biopsies were obtained from all cases. Pap smears and tumor biopsies were prepared for pathological confirmation.

HLA Typing

Genomic DNA was extracted from peripheral blood leukocytes using the QIAamp 96 DNA Blood kits (Qiagen, Venlo, the Netherlands). Sequence-based typing for the

Table 3. HLA-A-B Polymorphisms Among Cervical Squamous Cell Carcinoma (SCC) Patients and Controls

HLA-A-B	Case		Control		*P*	OR	95% CI
	Num	Frequency (%)	Num	Frequency (%)			
*001:01:01-37:01:01	2	1	7	1.38	1	0.72	0.15-3.51
01:01:01-57:01:01	4	2	3	0.59	0.107	3.44	0.76-15.49
02:01:01-13:01:01	2	1	3	0.59	0.625	1.7	0.28-10.25
02:01:01-40:01:01	**5**	**2.5**	**3**	**0.59**	**0.047**	**4.32**	**1.02-18.23**
02:01:01-46:01:01	**5**	**2.5**	**3**	**0.59**	**0.047**	**4.32**	**1.02-18.23**
02:01:01-51:01:01	2	1	1	0.2	0.196	5.12	0.46-56.8
02:01:01-56:01:01	**3**	**1.5**	**0**	**0**	**0.023**	**-**	**-**
02:03:01-38:02:01	6	3	13	2.56	0.952	1.18	0.44-3.14
02:03:01-51:01:02	2	1	0	0	0.081	-	-
02:06:01-51:01:01	0	0	9	1.77	0.069	0	-
02:07:01-40:01:01	3	1.5	4	0.79	0.412	1.92	0.43-8.65
02:07:01-46:01:01	19	9.5	49	9.65	1	0.98	0.56-1.72
11:01:01-13:01:01	6	3	13	2.56	0.952	1.18	0.44-3.14
11:01:01-15:02:01	12	6	27	5.31	0.875	1.14	0.56-2.29
11:01:01-40:01:01	10	5	26	5.12	1	0.98	0.46-2.06
11:01:01-46:01:01	4	2	6	1.18	0.481	1.71	0.48-6.12
11:01:01-51:01:01	0	0	9	1.77	0.069	0	-
11:01:01-51:01:02	3	1.5	1	0.2	0.072	7.72	0.8-74.67
11:01:01-54:01:01	2	1	6	1.18	1	0.85	0.17-4.22
11:01:01-55:02:01	2	1	5	0.98	1	1.02	0.2-5.28
11:01:01-56:03	2	1	3	0.59	0.625	1.7	0.28-10.25
11:01:01-67:01:01	2	1	1	0.2	0.196	5.12	0.46-56.8
11:02:01-40:01:01	2	1	4	0.79	0.678	1.27	0.23-7
11:02:01-46:01:01	2	1	2	0.39	0.32	2.56	0.36-18.27
24:02:01-13:01:01	5	2.5	6	1.18	0.359	2.15	0.65-7.11
24:02:01-15:02:01	2	1	4	0.79	0.678	1.27	0.23-7
24:02:01-35:01:01	3	1.5	4	0.79	0.412	1.92	0.43-8.65
24:02:01-40:01:01	6	3	11	2.17	0.716	1.4	0.51-3.83
24:02:01-51:01:01	0	0	8	1.57	0.114	0	-
24:02:01-55:02:01	4	2	3	0.59	0.107	3.44	0.76-15.49
29:01:01-07:05:01	2	1	1	0.2	0.196	5.12	0.46-56.8
30:01:01-13:02:01	3	1.5	12	2.36	0.576	0.63	0.18-2.25
31:01:02-51:01:02	2	1	0	0	0.081	-	-
33:03:01-44:03:02	0	0	7	1.38	0.2	0	-
33:03:01-58:01:01	14	7	43	8.46	0.658	0.81	0.43-1.52

-, Values are not calculated because of limited number of individuals in cases or controls; Num, Number of individuals; Only haplotypes with frequency of over 1% in cases or controls are listed; Haplotypes with significant difference as determined by chi-square test or Fisher exact test are showed in bold

three genes was performed by using SBT - AlleleSEQR Core Kits (Atria Genetics, South San Francisco, CA) on an ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). Sequences were analyzed by Assign version 3.5+ software (Conexio Genomics, Western Australia, Australia).

Statistical Analysis

The HLA A-B-DRB1 haplotypes were deduced for each individual using the Arlequin software (version 3.5) (Excoffier and Lischer, 2010). The frequency of participants with specific alleles or haplotypes was estimated. Only alleles with frequency over 1% in at least one group were used for subsequent statistical analysis using the R statistical software (version 2.15.1) analysis. Pearson chi-square test was used for the comparison of allele frequencies in the two groups. For cells in which the number of participants less than 5, two-tailed Fisher exact test was used. Calculated P values were corrected for multiple comparisons by the Benjamini and Hochberg method (Benjamini and Hochberg, 1995).

Results

All participants belonged to the Han population. At the study entry, the mean (standard deviation) and median ages of the cases were 45.03 (8.17) and 44.0 years old, respectively. The mean (standard deviation) and median ages of the controls were 43.05 (7.72) and 42.0 years old, respectively. None of the participants smoked cigarettes or drank alcohol.

A total of 28 alleles for HLA-A were genotyped. A*02 was the most common allele in both patients (33.5%) and controls (32.48%). The second most common allele was A*11. The frequencies of A*11 in patients and controls were 29.5% and 27.36%, respectively. According to the results of Pearson chi-square test or Fisher exact test, none of the alleles were significantly different in the two groups.

Table 1 shows the frequency distributions of HLA-B alleles. A total of 59 alleles were genotyped. B*40 was the most common alleles in both patients (18%) and controls (14.76%). The second most common allele was B*46:01:01 and its frequencies in patients and controls were 16.5% and 13.19%, respectively. Compared with the normal controls, cervical cancer patients had a significantly higher proportion carrying B*51:01:02 (5% vs 0.20%, $P = 3.17 \times 10^{-5}$, OR = 26.68), B*56 (3% vs 0.79%, $P = 0.037$, OR = 3.9) and B*56:01:01 (2% vs 0.20%, $P = 0.025$, OR = 10.35). The difference in B*51:01:02 allele frequency between cases and controls remained statistically significant ($P_c = 0.005$) after correction by the Benjamini and Hochberg method. The patients had a significantly lower frequency of B*44 (0%

Table 4. HLA-A-B-DRB1 Polymorphisms Among Cervical Squamous Cell Carcinoma (SCC) Patients and Controls

HLA-A-B-DRB1	Case		Control		*P*	OR	95% CI
	Num	Frequency (%)	Num	Frequency (%)			
01:01:01-37:01:01-10:01:01	2	1	7	1.377953	1	0.72	0.15-3.51
02:01:01-40:01:01-12:02:01	2	1	0	0	0.081	-	-
02:01:01-46:01:01-09:01:02	**5**	**2.5**	**2**	**0.393701**	**0.023**	**6.49**	**1.25-33.72**
02:01:01-58:01:01-03:01:01	2	1	1	0.19685	0.196	5.12	0.46-56.8
02:03:01-38:02:01-15:01:01	2	1	1	0.19685	0.196	5.12	0.46-56.8
02:03:01-38:02:01-16:02:01	3	1.5	8	1.574803	1	0.95	0.25-3.62
02:03:01-46:01:01-14:54	2	1	1	0.19685	0.196	5.12	0.46-56.8
02:07:01-46:01:01-08:03:02	4	2	3	0.590551	0.107	3.44	0.76-15.49
02:07:01-46:01:01-09:01:02	5	2.5	28	5.511811	0.148	0.44	0.17-1.15
02:07:01-46:01:01-12:02:01	2	1	3	0.590551	0.625	1.7	0.28-10.25
02:07:01-46:01:01-14:54	**5**	**2.5**	**1**	**0.19685**	**0.009**	**13**	**1.51-111.98**
11:01:01-13:01:01-15:01:01	2	1	7	1.377953	1	0.72	0.15-3.51
11:01:01-13:01:01-16:02:01	2	1	2	0.393701	0.32	2.56	0.36-18.27
11:01:01-15:02:01-12:02:01	9	4.5	14	2.755906	0.367	1.66	0.71-3.91
11:01:01-15:02:01-15:01:01	1	0.5	6	1.181102	0.68	0.42	0.05-3.51
11:01:01-35:01:01-15:01:01	2	1	1	0.19685	0.196	5.12	0.46-56.8
11:01:01-40:01:01-08:01:01	2	1	0	0	0.081	-	-
11:01:01-40:01:01-08:03:02	2	1	3	0.590551	0.625	1.7	0.28-10.25
11:01:01-40:01:01-09:01:02	2	1	6	1.181102	1	0.85	0.17-4.22
11:01:01-40:01:01-12:02:01	0	0	9	1.771654	0.069	0	-
11:01:01-40:01:01-13:12:01	2	1	0	0	0.081	-	-
11:01:01-46:01:01-09:01:02	4	2	4	0.787402	0.234	2.57	0.64-10.38
11:01:01-51:01:02-12:02:01	2	1	0	0	0.081	-	-
11:01:01-54:01:01-04:05:01	2	1	4	0.787402	0.678	1.27	0.23-7
11:02:01-40:01:01-16:02:01	2	1	2	0.393701	0.32	2.56	0.36-18.27
24:02:01-13:01:01-16:02:01	2	1	3	0.590551	0.625	1.7	0.28-10.25
24:02:01-15:02:01-12:02:01	2	1	5	0.984252	1	1.02	0.2-5.28
24:02:01-40:01:01-09:01:02	3	1.5	1	0.19685	0.072	7.72	0.8-74.67
24:02:01-40:01:01-15:01:01	1	0.5	6	1.181102	0.68	0.42	0.05-3.51
29:01:01-07:05:01-10:01:01	2	1	0	0	0.081	-	-
30:01:01-13:02:01-07:01:01	3	1.5	11	2.165354	0.767	0.69	0.19-2.49
31:01:02-51:01:02-16:02:01	2	1	0	0	0.081	-	-
33:03:01-44:03:02-07:01:01	0	0	6	1.181102	0.193	0	-
33:03:01-58:01:01-03:01:01	11	5.5	31	6.102362	0.911	0.9	0.44-1.82
33:03:01-58:01:01-13:02:01	0	0	6	1.181102	0.193	0	-

-, Values are not calculated because of limited number of individuals in cases or controls; Num, Number of individuals; Only haplotypes with frequency of over 1% in cases or controls are listed; Haplotypes with significant difference as determined by chi-square test or Fisher exact test are showed in bold

vs 3.35%, $P = 0.005$, OR = 0) and B*51:01:01 (1% vs 7.48%, $P = 7.01 \times 10^{-4}$, OR = 0.12). The difference in B*51:01:01 allele frequency between cases and controls remained statistically significant ($P_c = 0.03$) after multiple testing correction.

The frequency distributions of HLA-DRB1 are presented in Table 2. A total of 40 alleles were genotyped. DRB1*15 was the most common alleles in both patients (17%) and controls (15.95%). The second most common allele was DRB1*09 and its frequencies in patients and controls were 14.5% and 15.55%, respectively. The cervical cancer patients had a significantly higher proportion carrying DRB1*08 (9% vs 4.13%, $P = 0.027$, OR = 2.29), DRB1*08:01:01 (1.5% and 0%, $P = 0.023$) and DRB1*14:54 (5.5% vs 1.38%, $P = 0.006$, OR = 4.17). The percentage of DRB1*13:02:01 (0% vs 2.16%, $P = 0.04$, OR = 0) was significantly lower in patients than that in normal controls. None of the HLA-DRB1 alleles were significantly different in the two groups of subjects after multiple testing corrections.

A total of 192 HLA-A-B haplotypes were inferred and 35 of them were found to be with frequencies over 1% in at least one group of the participants. Detail information of these 35 haplotypes is presented in Table 3. HLA-A*02:07:01-B*46:01:01 was the most frequent haplotype in both SCC patients (9.5%) and controls (9.65%). The second most common haplotype in both groups was HLA-A*33:03:01-B*58:01:01 with the frequency of 7% and 8.46% in cases and controls, respectively. The cases had a significantly higher proportion carrying HLA-A*02:01:01-B*46:01:01 (2.5% vs 0.59%, $P = 0.047$, OR = 4.32), HLA-A*02:01:01-B*40:01:01 (2.5% vs 0.59%, $P = 0.047$, OR = 4.32), and HLA-A*02:01:01-B*56:01:01 (1.5% vs 0%, $P = 0.023$). None of the HLA-A-B haplotypes were significantly different in cases and controls after multiple testing corrections.

A total of 376 haplotypes were inferred and 35 of them were found to be with frequencies over 1% in at least one group of the participants. Detail information of these 35 haplotypes is presented in Table 4. HLA-A*33:03:01-B*58:01:01-DRB1*03:01:01 was the most frequent haplotype in both patients (5.5%) and healthy controls (6.10%). The second most common haplotype in patients was HLA-A*11:01:01-B*15:02:01-DRB1*12:02:01 with the frequency of 4.5%. The second most common haplotype in healthy controls was HLA-A*02:07:01-B*46:01:01-DRB1*09:01:02 (5.51%). The percentages of HLA-A*02:07:01-B*46:01:01-DRB1*14:54 (2.5% vs 0.20%, $P = 0.009$, OR = 13) and HLA-A*02:07:01-B*46:01:01-DRB1* 09:01:02 (2.5% vs 0.39%, $P = 0.023$, OR = 6.49) were significantly higher in patients than that in healthy controls. None of the HLA-A-B-DRB1 haplotypes were significantly different in cases and controls after multiple testing corrections.

Discussion

SCC is the most common type of cervical cancer (Howlader et al., 2013). It is a virally induced tumor, and immune response is likely to play important roles in the progression of this disease. In the present study, we investigated the associations of HLA-A, -B, and -DRB1 alleles with SCC on 100 SCC patients and 254 healthy control Chinese women.

Apart from the current study, there has been only one previous study on the association between HLA-A alleles and cervical cancer in Chinese and it reported that A*0207/0215N and A*2402 were associated with invasive cervical cancer in Chinese women from Hong Kong (Chan et al., 2005). However, our results showed that none the 28 HLA-A alleles in this study were significantly different in the two groups of participants. The inconsistence suggested that even within the same population, the HLA risk associations may vary among different regions. Similar variation has been observed in other HLA genes earlier. For example, a previous report (Wu et al., 2006) on the association between DQB1 alleles and cervical cancer in Chinese found a positive association of DQB1*060101 with cervical cancer, while this allele showed no difference in cases and controls in another study in Chinese women (Chan et al., 2007). Statistical analysis revealed a significantly increased frequency of B*51:01:02 ($P = 3.17 \times 10^{-5}$, $P_c = 0.005$, OR = 26.68) and a significantly decreased frequency of B*51:01:01 ($P = 7.01 \times 10^{-4}$, $P_c = 0.03$, OR = 0.12) in patients with SCC when compared with healthy controls. The result indicated that HLA-B*51:01:02 may confer susceptibility to SCC. And B*51:01:01 may be protective of SCC. These two alleles had not been reported previously. In addition, B*44 was found to have a nominally negative association with SCC ($P = 0.005$, OR = 0). This observation on Chinese women is in contrast to studies on Swedish (Zehbe et al., 2003) and Netherlandish (Bontkes et al., 1998) women, where B*44 alleles were found to associate with an increased risk for cervical cancer. This different host genetic background may be part of the reasons accounting for the differences in risk association observed for Chinese and European women. As for the HLA-DRB1 alleles, DRB1*13:02:01 was found with lower frequency among the SCC patients ($P = 0.04$, OR = 0). This is consistent with a previous report in Chinese women (Wu et al., 2007), where DRB1*13:02:01 were also found to be nominally protective of cervical cancer ($P = 0.013$, OR = 0.110, $P_c = 0.39$). Moreover, DRB1*13:02:01 has also been found to be with lower frequency among French (Sastre-Garau et al., 1996), Brazilian (Maciag et al., 2000) and other populations (Hildesheim et al., 1998; Beskow et al., 2001) with cervical cancer when compared with controls. Nominally positive associations with SCC were also found in DRB1*08, DRB1*08:01:01, and DRB1*14:54, which have not been reported before.

To our best knowledge, this is the first study to investigate the distribution HLA-A-B haplotype and HLA-A-B-DRB1 haplotype in Chinese women with SCC. Major HLA-A-B haplotypes with frequency over 5% in cases and controls were HLA-A*02:07:01-B*46:01:01, HLA-A*33:03:01-B*58:01:01, HLA-A*11:01:01-B*15:02:01, and HLA-A*11:01:01-B*40:01:01. None of these prevalent haplotypes were associated with SCC after correcting multiple testing. Only nominally association was detected in several haplotypes with frequencies less than 3%. Similar to HLA-A-B haplotypes, none of the prevalent HLA-A-B-DRB1 haplotypes were associated with SCC. This is consistent with a previous study of haplotype analysis in US and Costa Rica, which also reported that no HLA haplotype was significantly associated with cervical neoplasia (Carreon et al., 2005). This observation suggests that individual allele associations are independent of these underlying haplotypes.

In summary, our investigation suggests that HLA-B allele polymorphisms are involved in genetic susceptibility to SCC in Chinese women. In particular, statistical analysis revealed a significantly increased frequency of B*51:01:02 ($P = 3.17 \times 10^{-5}$, $P_c = 0.005$, OR = 26.68) and a significantly decreased frequency of B*51:01:01 ($P = 7.01 \times 10^{-4}$, $P_c = 0.03$, OR = 0.12) in patients with SCC when compared with healthy controls. These two alleles have not been reported to be associated with SCC before. None of the HLA-A-B or HLA-A-B-DRB1 haplotypes were associated with SCC after correcting multiple testing, implicating that individual allele associations are independent of the identified haplotypes.

Acknowledgements

The study was supported by Science and Technology Planning Project of Guangdong Province in China (2010B031600297). The author(s) declare that they have no competing interests.

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