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Corresponding Author:	Ruozheng Wang Xinjiang Medical University Affiliated Tumor Hospital CHINA
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Xinjiang Medical University Affiliated Tumor Hospital
Corresponding Author's Secondary Institution:	
First Author:	Mayinuer Alifu
First Author Secondary Information:	
Order of Authors:	Mayinuer Alifu
	Peiwen Fan
	Gulina kuerban
	Xuan Yao
	Yanchun Peng
	Tao Dong
	Ruozheng Wang
Order of Authors Secondary Information:	
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Abstract:	Purpose This study aims to investigate the association of human leukocyte antigen (HLA) alleles and haplotypes in Uyghur women with advanced squamous cell cervical cancer (SCC). Methods A total of 131 Uyghur patients with advanced SCC (IIb-IVa) and 91 healthy subjects from Xinjiang province were genotyped for HLA-I and II genes using Polymerase Chain Reaction Sequence Specific Primers (PCR-SSP). The different frequencies of HLA alleles and haplotypes between patients and controls were compared and the correlations were analyzed between HLA distribution and HPV status and prognosis. Results (1)The frequencies of B*51:01, DRB1*07:01, DQB1*02:01, A*01:01-C*06:02, A*01:01-DRB1*07:01, C*06:02-DQB1*02:01, DRB1*07:01-DQB1*02:01 and C*06:02-DRB1*07:01-DQB1*02:01 in cancer group were higher than control group whereas the frequencies of B*44:02, B*58:01, C*05:01, DRB1*04:01, DRB1*12:01, DRB1*13:01, DQB1*02:02, DQB1*05:02, DRB1*03:01-DQB1*02:02 and DRB1*04:01-DQB1*03:02

	in cancer group were lower than control group (P < 0.05). (2)The frequencies of A*01:01-C*06:02, A*01:01-DRB1*07:01, C*06:02-DQB1*02:01, DRB1*07:01-DQB1*02:01 and C*06:02-DRB1*07:01-DQB1*02:01 in HPV positive group were lower than HPV negative group, differences of which were statistically significant (P < 0.05). (3)B*44:02 and B*58:01 were associated with reduced DSS (P=0.010 and 0.007). (4)Multivariate Cox proportional hazard models revealed that age, FIGO stage, tumor differentiation and allele B*58:01 as independent predictors for DSS while FIGO stage and tumor differentiation as independent factors for DFS. Conclusions In the development and progression of advanced SCC among Uyghur population, the HLA alleles and its haplotypes play an important role. B*58:01 allele may act as independent predictor for DSS.
Other Authors:	Mayinuer Alifu
	Peiwen Fan
	Gulina kuerban
	Xuan Yao
	Yanchun Peng
	Tao Dong
Order of Authors (with Contributor Roles):	Mayinuer Alifu (Data curation: Lead; Formal analysis: Lead; Methodology: Equal; Resources: Lead; Writing – original draft: Lead; Writing – review & editing: Lead)
	Peiwen Fan (Data curation: Equal; Methodology: Equal; Writing – original draft: Equal; Writing – review & editing: Equal)
	Gulina kuerban (Data curation: Supporting; Methodology: Equal; Project administration: Supporting; Resources: Lead; Writing – original draft: Equal; Writing – review & editing: Equal)
	Xuan Yao (Formal analysis: Supporting; Methodology: Supporting; Writing – original draft: Supporting; Writing – review & editing: Lead)
	Yanchun Peng (Formal analysis: Supporting; Methodology: Supporting; Writing – review & editing: Lead)
	Tao Dong (Conceptualization: Lead; Data curation: Lead; Methodology: Lead; Project administration: Lead; Supervision: Lead; Validation: Lead; Writing – review & editing: Lead)
	Ruozheng Wang (Conceptualization: Lead; Data curation: Lead; Formal analysis: Lead; Funding acquisition: Lead; Investigation: Lead; Methodology: Lead; Project administration: Lead; Supervision: Lead; Validation: Lead; Writing – review & editing: Lead)

Frequency distribution of HLA alleles and haplotypes in Uyghur women with advanced squamous cell cervical cancer and relation to HPV status and clinical outcome

Mayinuer Alifu[1,*], Peiwen Fan[2,*], Gulina kuerban[3], Xuan Yao[4], Yanchun Peng[4],Tao Dong[4], Ruozheng Wang[1,2]

[1]Department of Radiation Oncology, the Affiliated Tumor Hospital of Xinjiang Medical University, Urumqi, Xinjiang 830000, China

[2]Key Laboratory of Oncology in Xinjiang Uyghur Autonomous Region, Urumqi, Xinjiang 830000, China

[3]Department of Gynecology, the Affiliated Tumor Hospital of Xinjiang Medical University, Urumqi, Xinjiang 830000, China

[4]MRC Human Immunology Unit, the Weatherall Institute of Molecular Medicine, University of Oxford, Oxford OX3 9DS, UK

*These authors have contributed equally to this work

Correspondence to: Ruozheng Wang, E-mail: wrz8526@vip.163.com

Tao Dong, E-mail:tao.dong@imm.ox.ac.uk

Abstract

Purpose This study aims to investigate the association of human leukocyte antigen (HLA) alleles and haplotypes in Uyghur women with advanced squamous cell cervical cancer (SCC).

Methods A total of 131 Uyghur patients with advanced SCC (IIb-IVa) and 91 healthy subjects from Xinjiang province were genotyped for HLA-I and II genes using Polymerase Chain Reaction Sequence Specific Primers (PCR-SSP). The different frequencies of HLA alleles and haplotypes between patients and controls were compared and the correlations were analyzed between HLA distribution and HPV status and prognosis.

Results (1)The frequencies of B*51:01, DRB1*07:01, DQB1*02:01, A*01:01-C*06:02, A*01:01-DRB1*07:01, C*06:02-DQB1*02:01, DRB1*07:01-DQB1*02:01 and C*06:02-DRB1*07:01-DQB1*02:01 in cancer group were higher than control group whereas the frequencies of B*44:02, B*58:01, C*05:01, DRB1*04:01, DRB1*12:01, DRB1*13:01, DQB1*02:02, DQB1*05:02, DRB1*03:01-DQB1*02:02 and DRB1*04:01-DQB1*03:02 in cancer group were lower than control group ($P<0.05$). (2)The frequencies of A*01:01-C*06:02, A*01:01-DRB1*07:01, C*06:02-DQB1*02:01, DRB1*07:01-DQB1*02:01 and C*06:02-DRB1*07:01-DQB1*02:01 in HPV positive group were lower than HPV negative group, differences of which were statistically significant ($P<0.05$). (3)B*44:02 and B*58:01 were associated with reduced DSS ($P=0.010$ and 0.007). (4)Multivariate Cox proportional hazard models revealed that age, FIGO stage, tumor differentiation and allele B*58:01 as independent predictors for DSS while FIGO stage and tumor differentiation as independent factors for DFS.

Conclusions In the development and progression of advanced SCC among Uyghur population, the HLA alleles and its haplotypes play an important role. B*58:01 allele may act as independent predictor for DSS.

Key words Squamous cell cervical cancer (SCC), Human leukocyte antigen (HLA), Haplotype, Uyghur, Clinical outcome

Introduction

Uterine cervical cancer (CaCx) is a public health problem [1]. It is the third most commonly diagnosed cancer and the fourth leading cause of cancer death among females worldwide, imposing especially high burden in developing countries mainly due to the absence of efficient screening programs [2] and limited or no access to treatment for the patients. In China, CaCx is currently the leading gynecological malignancy in women [3]. According to the cancer statistics in China, it is predicted that there will be about 98.9 thousand newly diagnosed CaCx and 30.5 thousand deaths in 2015 [4], suggesting that a substantial increase in CaCx incidence will be seen in China. Xinjiang Uyghur Autonomous Region is located in the northwest of the People's Republic of China and Uyghur ethnic is one of the indigenous people in the Xinjiang, China [5]. The incidence of CaCx among Uyghur women is four times higher than the mean incidence of China [6]. They have the highest morbidity and mortality resulted from CaCx in the country, even when compared with women of other ethnicities from the same geographic area [7]. When detected at an early stage, invasive CaCx is one of the most treatable cancers. The 5-year CaCx survival rate ranges from 60 to 70% in most countries [8]. However, most of the Uyghur females with CaCx are already in the middle or late stage when they were admitted into the hospital, missing the chance of surgery, about 80% of patients of Uyghur ethnicity present at an advanced stage [9]. Limited access to CaCx screening and treatment services like this contributes to a disproportionate rate of morbidity and mortality for them. Therefore, there was important social value to research on the occurrence and prognosis of cervical lesions in Xinjiang Uyghur women [5].

CaCx is almost exclusively (99%) caused by a persistent infection with human papillomavirus (HPV) infections [10]. Squamous cell carcinoma (SCC) is the most common histological type of cervical cancer and it develops from the cells that line the inner part of the cervix, Immune response is believed to play important roles in the progression of it [11-12]. More than 90% of SCC of the cervix contain DNA from specific high-risk types of HPV (HR-HPV), which are sexually transmitted [13]. Experimental and clinical evidences demonstrate that the immunological and genetic backgrounds of the host play an important role in the outcome of HPV associated infection. The activation of an effective immune response against virus-infected and tumor cells involve the antigen presentation to specific T cells [14]. Host genetic factors, especially human leukocyte antigen (HLA) genes with an extraordinary degree of polymorphism have an important role in HPV infection and further contribute to the progression to CaCx [15]. Class I HLA molecules (HLA-A, B, and C) present foreign antigens to $CD8^+$ cytotoxic T lymphocytes, and class II molecules (HLA-DR and DQ) present antigenic peptides to $CD4^+$T helper cells [16].

Considering the important role of HLA in the immune system, several studies have been conducted to look into associations between CaCx and HLA gene distribution in Xinjiang population [17]. However, the relationship between HLA class I and II polymorphisms and the development of CaCx among Uyghur women still remain to be uncovered. Moreover, there is no report available on characterizing HLA haplotypes in Uyghur patients with CaCx. In this study, we carried out high-resolution HLA typing to evaluate the risk of advanced SCC (IIb-IVa) of Uyghur women in association with class I HLA-A, -B, and -C and class II HLA-DRB1 and -DQB1 loci. Also, HLA alleles and haplotypes were analyzed in relation to tumor HPV status and clinical outcome.

Materials and methods

Study subjects

A total of 131 Uyghur patients who were histologically confirmed with SCC, International Federation of Gynaecology and Obstetrics (FIGO) stage IIb-IVa, Karnofsky performance status point ≥80 and underwent a radiotherapy and/or chemotherapy as primary treatment at the Affiliated Tumor Hospital of Xinjiang Medical University from May 2012 to March 2016 were enrolled in this study. Meanwhile, a total of 91 healthy women without a family history of CaCx from the physical examination center of the same hospital were enrolled in the control group.

Clinical data and follow-up

General clinical data like age, FIGO stage, tumor differentiation and treatment history were obtained by referring to the hospital electronic medical records. All of the patients were treated with radiotherapy and/or chemotherapy. Intracavitary and/or extracorporeal irradiation were used according to the patient's specific condition. PVB (cisplatin 50 mg/m2 + bleomycin hydrochloride 30 mg/m2 + vincristine sulfate 1.5 mg/m2) or TP (taxol 135 mg/m2 + cisplatin 50 mg/m2) was applied to the patients according to their conditions. The total chemotherapy cycles were three times. After completion of the therapy, patients were followed at 3-month intervals during the first 2 years and at 6-month intervals thereafter. Survival and recurrence data were collected for all patients. Disease-specific survival (DSS) was defined as the time from diagnosis to death due to SCC. Disease-free survival (DFS) was defined as time from diagnosis until the date of first disease recurrence (local, regional, distant or a combination).

HPV typing

Vaginal secretions were taken from all patients to determine the HPV status. Two gynecologic worked together to exposed the cervix of the uterus and took samples using disposable cervical cell collector (Chaozhou Kaipu Biochemistry Co. Ltd.,). HPV subtype in samples was determined by HybriMax HPV DNA detection method.

HLA Genotyping

Peripheral bloods were collected in vacuum tubes containing EDTA anticoagulant. The genomic DNA was extracted from whole blood by strictly following the manufacturer's instructions of blood genomic DNA extraction kits (Bioteke Corp., China). Ultraviolet spectrophotometer (Thermo Fisher ScientifcInc.,Waltham, MA, USA) was used to determine the concentration and purity of the DNA. The range of A260/280 ratios was 1.8-1.9.The final concentration of each DNA sample was adjusted to 0.3–0.5μg/μL and stored in -20°Cfreezer until the test. HLA alleles were genotyped using the polymerase chain reaction with sequence-specific primers [17].

Statistical analysis

SPSS software (version 21.0, Chicago, IL, USA) was used for the statistical analyses. Chi-square or two-tailed Fisher's exact test was performed to compare the allele frequencies in patients and control groups. Alleles present in 4% or more of the case or control subjects were included in the main single locus analyses and co-occurring alleles to have the analysis with meaningful sample sizes [18]. The relative risks were calculated by the Odds ratios (ORs) with 95% confidence intervals (*CI*s). Kaplan–Meier method and the log-rank test were used to compare the DSS and DFS. To examine risk, univariate and multivariate COX regression analysis were performed. $P<0.05$ was regarded as

statistically significant in two sided tests.

Results

Mean age at diagnosis of patients was 52.7±9.8years (range32 to 73) while controls was 50.2±8.7years (range from 29 to 71). Our follow-up ended in July 2017 and we have lost 8 patients because the phone number was wrong or had been stopped (the missing follow-up rate was 6.1%). One patient died of intestinal obstruction during treatment. Finally, follow-up data from 122 patients were obtained. Clinical detail information of 131 advanced SCC patients is shown in Table 1.

Table 1 General clinical data of 131 advanced SCC patients investigated

Factor	N	Constituent ratio（%）
FIGO stage		
IIb	70	53.4
IIIa-IIIb	57	43.5
IVa	4	3.1
Tumor differentiation		
Well	9	6.9
Moderate	101	77.1
Poor	21	16.0
HPV status		
Positive	120	91.6
Negative	11	8.4
HPV16		
Positive	97	74.0
Negative	34	26.0
Treatment		
Radiotherapy	49	37.4
Radiotherapy+chemotherapy	82	62.6
Death		
Yes(SCC)	34	25.9
No	88	67.2
Missing or others	9	6.9
Disease recurrence		
Yes	32	24.4
No	91	69.5
Missing	8	6.1

SCC: squamouscell cervical cancer; N: number of patients; FIGO: International Federation of Gynecology and Obstetrics; HPV: human papillomavirus.

Distribution of HLA alleles and haplotypes

The HLA 31 A, 51 B, 26 C, 38 DRB1 and 18 DQB1 alleles were identified with high resolution methods. Alleles which were presented over 4% of the cancer patients or subjects from control groups were included in the main single-locus and haplotypes analysis (Only statistically significant data were shown in Figure1). Among HLA class I alleles, a total of 31 alleles that＞4% were genotyped (9

A, 10 B and 12 C). The most common HLA-A in cancer group was A*01:01 with the frequency of 13.4% while A*03:01 was the most common allele in control group with the frequency of 13.7%. However, no significant association was observed for HLA-A alleles with SCC. HLA-B*51:01 was the most common allele in both SCC patients and controls (15.6% VS 8.2%), and its presence was observed to be associated with increased risk for SCC (P=0.021, OR=2.065). The second most common alleles were B*50:01 in cancer group (7.3%) and B*13:02 in control group (7.1%), but none of the allele frequencies were significantly different in the two groups (P=0.133 and P=0.664). The third most common allele was B*35:03 in cancer group (6.5%) while B*44:02 and B*58:01 were the third common alleles in control group, both with frequency at 5.5%. Also, B*44:02 (P=0.017, OR=0.199) and B*58:01 (P=0.040, OR=0.335) were associated with decreased risk for SCC. HLA-C*06:02 and C*07:02 were the most common and the second most common alleles in both SCC patients and controls (18.3% VS 14.8 and 12.2% VS 9.9%), but there was no statistically significant difference in the frequencies of these alleles (P=0.335 and P=0.446). Besides, C*05:01 was the sixth common allele in control group (6.0%) and least common allele in cancer group (1.5%), which was inferred to confer decreased risk associated with SCC (P=0.020, OR=0.241).

Among HLA class II alleles, a total of 19 alleles which were presented in over 4% subjects were genotyped (10 DRB1 and 9 DQB1). HLA-DRB1*07:01 was the most common allele in both SCC patients and control subjects (25.6% and 12.6%). Compared to controls, SCC patients had a significantly higher chance of carrying this allele, suggesting its role to increase susceptibility for SCC (P=0.001, OR=2.375). The second common allele in controls was DRB1*04:01, the percentage of which was significantly higher in control group (11.0%) than cancer group (4.6%), indicating a potential protective effect for SCC (P=0.010, OR=0.389). In DRB1, two other alleles, DRB1*12:01 and DRB1*13:01, were found to be associated with decreased risk of SCC (P=0.004, OR=0.164 and P=0.047, OR=0.442). On the other hand, as for HLA-DQB1, the most enriched allele in SCC patients was DQB1*02:01 (21.0%) while the most common allele in controls was DQB1*03:01 (18.1%). A significantly elevated frequency in DQB1*02:01 allele was found in SCC patients as compared with controls (7.1%, $P<0.001$, OR=3.454). DQB1*02:02 and DQB1*05:02 were the second and fourth common alleles in controls (13.2% and 11.0%). As compared to SCC patients (6.5% and 5.7%), these alleles were likely to serve a protective role for SCC (P=0.016, OR=0.457 and P=0.043, OR=0.492).

In multilocus analysis, there were only 7 haplotypes occurring in over 4% of the SCC patients or controls. The most common haplotype in cancer group was DRB1*07:01-DQB1*02:01 (10.7%). A positive association was observed for this haplotype with SCC ($P<0.001$, OR=3.840) which suggested it as a susceptible haplotype for SCC. The most common haplotype in control group was C*06:02-DRB1*07:01 (6.6%). However, no statistically significant differences was found in its frequency when compared to that in cancer patients (9.4%, P=0.141). Meanwhile, statistical analysis demonstrated significantly increased frequencies of two-locus haplotypes such as A*01:01-C*06:02 (P=0.009, OR=3.115), A*01:01-DRB1*07:01 ($P<0.001$, OR=17.424) and C*06:02-DQB1*02:01 (P=0.027, OR=2.157) in SCC group when compared with control group, indicating that these haplotypes may confer susceptibility to SCC. Furthermore, a three-locus haplotype C*06:02-DRB1*07:01-DQB1*02:01 was also found to be associated with a higher risk for SCC (4.5% VS 1.5%, P=0.001, OR=3.060). The lower frequency of two-locus haplotypes DRB1*03:01-DQB1*02:02 and DRB1*04:01-DQB1*03:02 in SCC group suggested a protective role of this haplotype ($P<0.001$, OR=0.044 and P=0.001, OR=0.236).

Fig.1 Statistically significant alleles and haplotypes in SCC patients and control subjects. The frequencies of eleven alleles and seven haplotypes were significantly different between SCC patients and control individuals, the *P* values for all alleles and haplotypes were less than 0.05.

HPV status and clinical outcome

We investigated the relationship between HLA frequency and HPV status in 131 advanced SCC patients and found out that five haplotypes were correlated with HPV status (Table2 and Figure 2, N=131). The frequencies of these haplotypes in HPV positive group were significantly lower than HPV negative group ($P<0.05$). Also, a weak association was observed between the two-locus haplotype A*01:01-DRB1*07:01 and the HPV16 status (3.6% VS 7.4%, $P=0.072$).

Table 2 The frequencies of HLA alleles and haplotypes in HPV status

HLA trait (alleles or haplotypes)	HPV			HPV 16		
	Pos	Neg	*P*	Pos	Neg	*P*
B*44:02	3(1.3)	0(0.0)	1.000	1(0.5)	2(2.9)	0.339
B*51:01	39(16.3)	2(9.1)	0.563	32(16.5)	9(13.2)	0.524
B*58:01	5(2.1)	0(0.0)	1.000	4(2.1)	1(1.5)	1.000
C*05:01	4(1.7)	0(0.0)	1.000	2(1.0)	2(2.9)	0.596
DRB1*04:01	11(4.6)	1(4.5)	1.000	8(4.1)	4(5.9)	0.795
DRB1*07:01	58(24.2)	9(40.9)	0.085	49(25.3)	18(26.5)	0.844
DRB1*12:01	3(1.3)	0(0.0)	1.000	3(1.5)	0(0.0)	0.570
DRB1*13:01	10(4.2)	0(0.0)	1.000	9(4.6)	1(1.5)	0.420
DQB1*02:01	49(20.4)	6(27.3)	0.450	44(22.7)	11(16.2)	0.257
DQB1*02:02	15(6.3)	2(9.1)	0.948	10(5.2)	7(10.3)	0.139
DQB1*05:02	13(5.4)	2(9.1)	0.818	9(4.6)	6(8.8)	0.201
A*01:01-C*06:02	21(4.4)	5(11.4)	0.041*	18(4.6)	8(5.9)	0.566
A*01:01-DRB1*07:01	19(4.0)	5(11.4)	0.025*	14(3.6)	10(7.4)	0.072
C*06:02-DQB1*02:01	26(5.4)	7(15.9)	0.006*	25(6.4)	8(5.9)	0.817
DRB1*03:01-DQB1*02:02	1(0.2)	0(0.0)	1.000	0(0.0)	1(0.7)	0.260
DRB1*04:01-DQB1*03:02	6(1.3)	0(0.0)	1.000	5(1.3)	1(0.7)	0.957
DRB1*07:01-DQB1*02:01	46(9.6)	10(22.7)	0.007*	43(11.1)	13(9.6)	0.621
C*06:02-DRB1*07:01-DQB1*02:01	34(3.5)	13(14.8)	0.000*	33(4.3)	14(5.1)	0.540

HLA: human leukocyte antigens; HPV: human papillomavirus; Pos: positive; Neg: negative; *: $P<0.05$.

Fig.2 The frequencies of HLA alleles and haplotypes in HPV status. The frequencies of two-locus haplotypes such as A*01:01-C*06:02, A*01:01-DRB1*07:01, C*06:02-DQB1*02:01 and DRB1*07:01-DQB1*02:01, and three-locus haplotypes, C*06:02-DRB1*07:01-DQB1*02:01, in HPV positive group were significantly lower than HPV negative group, the *P* values of them were less than 0.05.

A total of 122 patients were followed and selected as subjects of the survival analysis. Among them, the median follow-up was 25 months (range 3 to 53 months). Only three patients carried the B*44:02 allele while two of them died of SCC. Of the 119 patients who did not carry the B*44:02

allele, thirty two died of SCC. The DSS of B*44:02 carrier and non-carrier were 33.3% and 73.1%, respectively. B*44:02 carriers displayed a lower DSS (P=0.010). Meanwhile, three out of the five patients carrying the B*58:01 allele and died of SCC. For those who did not carry the B*58:01 allele, 31 died of SCC. The DSS of B*58:01 carrier and non-carrier were 40.0% and 73.5%, respectively. The presence of B*58:01was inversely correlated with DSS, similar to that of B*44:02 (P=0.007). Survival curves of the B*44:02 and B*58:01were showed in Figure3. No significant difference was found in DFS when comparing subjects carrying different HLA alleles or haplotypes carrier and non-carrier (data not shown).

Fig.3 Disease-specific survival (DSS) in 122 patients with advanced SCC. a Kaplan-Meier curves HLA-B*44:02 carrier and non-carrier (log-rank test, P=0.010). b Kaplan-Meier curves B*58:01 carrier and non-carrier (log-rank test, P=0.007). The patients of B*44:02 and B*58:01 carrier displayed poor DSS compared to non-carrier patients.

Univariate cox analysis was performed to evaluate the impact of 11 alleles and 7 haplotypes on DFS and DSS in patients with advanced SCC. Clinicopathological parameters such as age, FIGO stage, tumor differentiation, HPV and HPV16 status were also analyzed to determine the HR of each variable. Considering multiple combinations of HLA alleles or haplotypes and clinicopathological parameters, we used statistically significant factors in univariate as covariates in multivariate COX proportional hazards model (Only statistically significant factors in univariate and multivariate COX regression were listed in Table 3). The univariate analysis results showed that age, FIGO stage, tumor differentiation, B*44:02 and B*58:01 were associated with poor DSS. Significantly poorer DFS was found in patients with advancing FIGO stage and poor tumor differentiation, while no HLA alleles or haplotypes linked to DFS. Multivariate COX regression results showed that two critical prognostic factors, FIGO stage and tumor differentiation, in our study population were considered as independent risk factors for DSS and DFS. Only one allele, HLA-B*58:01, was an independent risk factor for DSS while age was another independent prognostic factor that affected DSS.

Table 3 Univariate and multivariate COX regression of HLA alleles and clinicopathological parameters on DSS and DFS

DSS	P_{coxuni}	HR_{coxuni}(95% CI)	P_{coxmul}	$HR_{coxmuli}$(95% CI)
Age	0.016*	1.044(1.008-1.082)	0.007*	1.051(1.013-1.090)
FIGO stage	0.003*	2.311(1.322-4.039)	0.001*	2.838(1.524-5.283)
Tumor differentiation	0.016*	2.469(1.184-5.148)	0.010*	2.546(1.256-5.162)
B*44:02	0.023*	5.501(1.268-23.872)	-	-
B*58:01	0.014*	4.506(1.357-14.967)	0.011*	4.969(1.443-17.115)
DFS	P_{coxuni}	HR_{coxuni}(95% CI)	P_{coxmul}	$HR_{coxmuli}$(95% CI)
FIGO stage	0.000*	3.499(1.944-6.297)	0.000*	3.739(2.002-6.912)

Tumor differentiation	0.022*	2.492(1.141-5.443)	0.015*	2.575(1.201-5.519)

HLA: human leukocyte antigens; DSS: disease-specific survival; DFS: disease free survival; P_{coxuni} and P_{coxmul}: *P* value of univariate and multivariate COX regression; HR_{coxuni} and HR_{coxmul}: hazard ratio of univariate and multivariate COX regression; CI: confidence interval; FIGO: International Federation of Gynecology and Obstetrics; 95% *CI:* 95% confidence intervals; *: $P<0.05$.

Discussion

In this Uyghur population-based study, there were 11 HLA alleles (4 HLA class I and 7 HLA class II) and 7 haplotypes (6 two-locus and 1 three-locus haplotypes) being proved to be significantly associated with advanced SCC. We believe that this study is the first one to report the study conducted among Uyghur patients with SCC, in terms of HLA frequency distribution and the relationship between HPV status and patient survival in cervical cancer.

Previous studies have found that HLA-A genes were involved in the development of SCC [19, 20]. However, Gonzales-Galarza et al. [21] indicated that the comparison of HLA-A allele frequencies between the family sample and neighbouring populations did not reveal any significant difference. Similar to this, none of the 9 HLA-A alleles in our study were significantly different between SCC patients and controls. A family study based on the Croatian population reported that B*51:01 is the most frequent among HLA-B alleles, as well as among some other populations on the European South [22]. The B*51:01 is also the most frequent allele in Asians, including Uyghur [23]. And, one study carried among the population of Chinese women from Han people reported that B*51:01:02 may confer susceptibility to SCC while B*51:01:01 may be protective of SCC [11]. In our study, B*51:01 was also the most common allele in both SCC patients and controls, and observed to be associated with increased risk for SCC. HLA-B*58:01 allelic frequency is much higher in Asians (10-15%) than Europeans (1-3%) [24] and was reported as an increased risk factor for Indian women (OR=8.26) [16]. However, compared with controls, a significantly decreased frequencies of B*58:01 in Uyghur SCC patients were observed in our study (OR=0.335). At the same time, B*44:02 and C*05:01 were found to be significantly linked to elevated risks of SCC (OR=1.9 and 1.6) [19]. However, our study told otherwise. Decreased frequencies of B*44:02and C*05:01 in SCC patients were observed in our study. The main reason might be that the predominant genetic variants in different population are not the same, suggesting that there may be different risk factors associated with SCC for Uyghur people and that of Chinese Han people, American or Indian women.

As for HLA class II alleles, The DRB1*07:01 was found to be the most frequent allele in Uyghur ethnic group, as well as in some other populations [23]. Yang et al. [25] report that DRB1*07:01 tended to confer an elevated risk of SCC (OR=2.89). An increased risk for cervical cancer was also reported for the allele DRB1*07:01 in other two studies [18, 26]. In our study, DRB1*07:01 was found to displaying higher frequency among SCC patients when comparing with controls (OR=2.375), which indicated that the allele may confer increased susceptibility to SCC. This is consistent with their results. A meta-analysis on the relationship between HLA-DRBl gene polymorphism and cervical cancer in Chinese population demonstrated that there was no significant difference between the control groups of the various studies for DRB1*04:01 [27]. However, this meta-analysis study did not

find any association between specific alleles with cervical cancer in Uyghur population. Cuzick et al. [28] pointed out that DRB1*04:01 was a risk factor in British populations. On contrary, DRB1*04:01 showed protective effect for Uyghur women with SCC in our study. This might explain HLA allele frequencies differ extensively among various populations. On the other hand, there are several studies reported that DRB1*12:01 and/or *13:01 alleles are associated with a reduced cervical cancer risk [18, 29-30]. Further analysis at DRB1 locus in our study revealed that DRB1*12:01 and *13:01 showed protective effect for Uyghur women with SCC. HLA-DQB1*02:01 (18.9%) and*05:01 (13.8%) were frequent alleles in Yazd province population [31]. Similar to this, DQB1*02:01 was the most common allele in Uyghur population in our study with the frequency of 21.0%, and was found potential susceptible gene for SCC (OR=3.454). Gokhale et al. [18] reported that DQB1*02:01 was also found to be related to an increased risk for cervical cancer in Indian women. However, Rathika et al. [26] pointed out that there was no statistically significant difference between cancer patients and controls regarding the frequency of DQB1*02:01 and *02:02 alleles. On the contrary, DQB1*02:02 acted as a protection allele with SCC among Uyghur women. The results from one study conducted among Chinese Han women revealed that DQB1*05:02 was the protective alleles for cervical cancer [32]. This is consistent with our result. We also find out that DQB1*05:02 might provide protection to cervical cancer in Uyghur women. The characteristics of cervical cancer and the overall distribution of HLA in Xinjiang Uyghurs are not the same as in Han Chinese and other ethnic groups, these findings suggest a potential effect of genetic predisposition among races [33].

The knowledge of HLA allele and haplotype distribution is very useful in the treatment of CaCx. Thus, our study also discovered that increased frequencies of several two-locus haplotypes, A*01:01-C*06:02， A*01:01-DRB1*07:01, C*06:02-DQB1*02:01, DRB1*07:01-DQB1*02:01, and one three-locus haplotype, C*06:02-DRB1*07:01-DQB1*02:01, existed in cervical cancer patients, implying a susceptible role of these haplotypes. On the contrary, the haplotypes DRB1*03:01-DQB1*02:02 and DRB1*04:01-DQB1*03:02 conferred decreased risk for cervical cancer. This is the first study to investigate the distribution HLA class I and II in Uyghur women with SCC. In the previous studies conducted in other labs, we did not find haplotypes which displayed completely the same association with cervical cancer as that uncovered by ours. What has been reported is that DRB1*04:01-DQB1*03:01 [26] was associated with increased risk whereas DRB1*07:01-DQB1*02:02 [18] was associated with decreased risk for cervical cancer.

As we know, CaCx is a typical type of cancer related to virus infection. HPV infection is a premise for the onset and development of cervical cancer. It is possible that genetic factors influence viral persistence, and specific more virulent variants of HPV may play important roles in early onset cervical SCCs [34]. Indeed, many viral infections (including HPV) are controlled by T cells restricted to HLA. The HLA-DRB1*15:01 and HLA-DRB1*15:01-DQB1*06:02 haplotypes inhibit Han invasive cervical cancer (ICC) cell antigens presented to CD4+ T cells and decrease the risk of HPV infection and ICC occurrence while HLA-DQB1*06:02 is involved in protecting Uyghur cervical tissues from HPV16 infection and cervical cancer occurrence [17]. To better understand of HLA distributions related to in Uyghur SCC patients, we divided patients with different HPV status into positive and negative groups, and analyses the different frequencies of HLA alleles and haplotypes in the two groups. We found that frequencies of susceptible haplotypes A*01:01-C*06:02, A*01:01-DRB1*07:01, C*06:02-DQB1*02:01, DRB1*07:01-DQB1*02:01 and C*06:02-DRB1*07:01-DQB1*02:01 in HPV positive group were lower than that in HPV negative

group ($P<0.05$). This may partly explain the reason why the women who not infected with HPV are also likely to develop cervical cancer. However, there is still need for further study to validate on this hypothesis.

We were absorbed in the distribution of HLA alleles and haplotypes as well as focused on their association with HPV infection in this study. In addition, we evaluated the relationship between HLA distribution and clinical outcome of SCC. Results of univariate and multivariate COX regression analysis indicated that FIGO stage, tumor differentiation and allele HLA-B*58:01were independent risk factors for DSS. When cancer patients and control subjects are compared, B*58:01 may be a protective allele for Uyghur women. However, multivariate COX regression told the opposite. This might suggest that other factors could function together with this allele to affect the onset and development of cervical cancer. This hypothesis deserved further study involving more clinical information.

In summary, for the first time our study reported the frequency distribution of HLA alleles and haplotypes in Uyghur women with advanced SCC. It is also found that the HLA gene distribution is related to HPV status and clinical outcome. However, there are still a few more questions to be solved in further study. Firstly, controls in this study only included women who uninfected with HPV. It would be more interesting to see if HLA alleles distributed differently between HPV-infected subjects with or without cervical cancer. Secondly, since it is well acknowledged that ethnicity is one of the factors affecting HLA gene distribution, it would be worthwhile to compare the association of HLA gene distribution and the risk of SCC development among women from different ethnic background but living in the same area, such as Han women. Last but not least, expanding the sample size will help to test the hypothesis generated in our current study. Better still we hope to investigate the HPV protein-specific T cells to uncover the interplay between the HLA molecules and immune system, so as to provide evidence on the molecular and cellular level to explain the role of HLA in HPV-related SCC development.

Conclusion

Our findings indicated that HLA class I and II alleles and haplotypes affect the occurrence and development of advanced SCC in Uyghur population. FIGO stage, tumor differentiation and allele HLA-B*58:01were independent risk factors for DSS. However, there still need to validate by expanding the sample size explain the reason. The results suggested that HLA class I and II polymorphisms are potentially involved into the onset and development of SCC in Uyghur women.

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Author contribution TD and RZW: Protocol/project development, data collection or management, data analysis, manuscript editing. MA, PWF and GK: data collection or management, data analysis, manuscript writing/editing. XY and YCP: data analysis, manuscript editing.

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Compliance with Ethical Standards

Conflicts of interests All authors declared that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in

accordance with the ethical standards of Xinjiang Medical University Affiliated Tumor Hospital ethics Committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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Alifu M: Data collection or management, data analysis, manuscript writing/editing.

Fan PW: Data collection or management, data analysis, manuscript writing/editing.

Kuerban G: Data collection or management, data analysis, manuscript writing/editing.

Yao X: Data analysis, manuscript editing

Peng YC: Data analysis, manuscript editing

Dong T: Protocol/project development, data collection or management, data analysis, manuscript editing.

Wang RZ: Protocol/project development, data collection or management, data analysis, manuscript editing.

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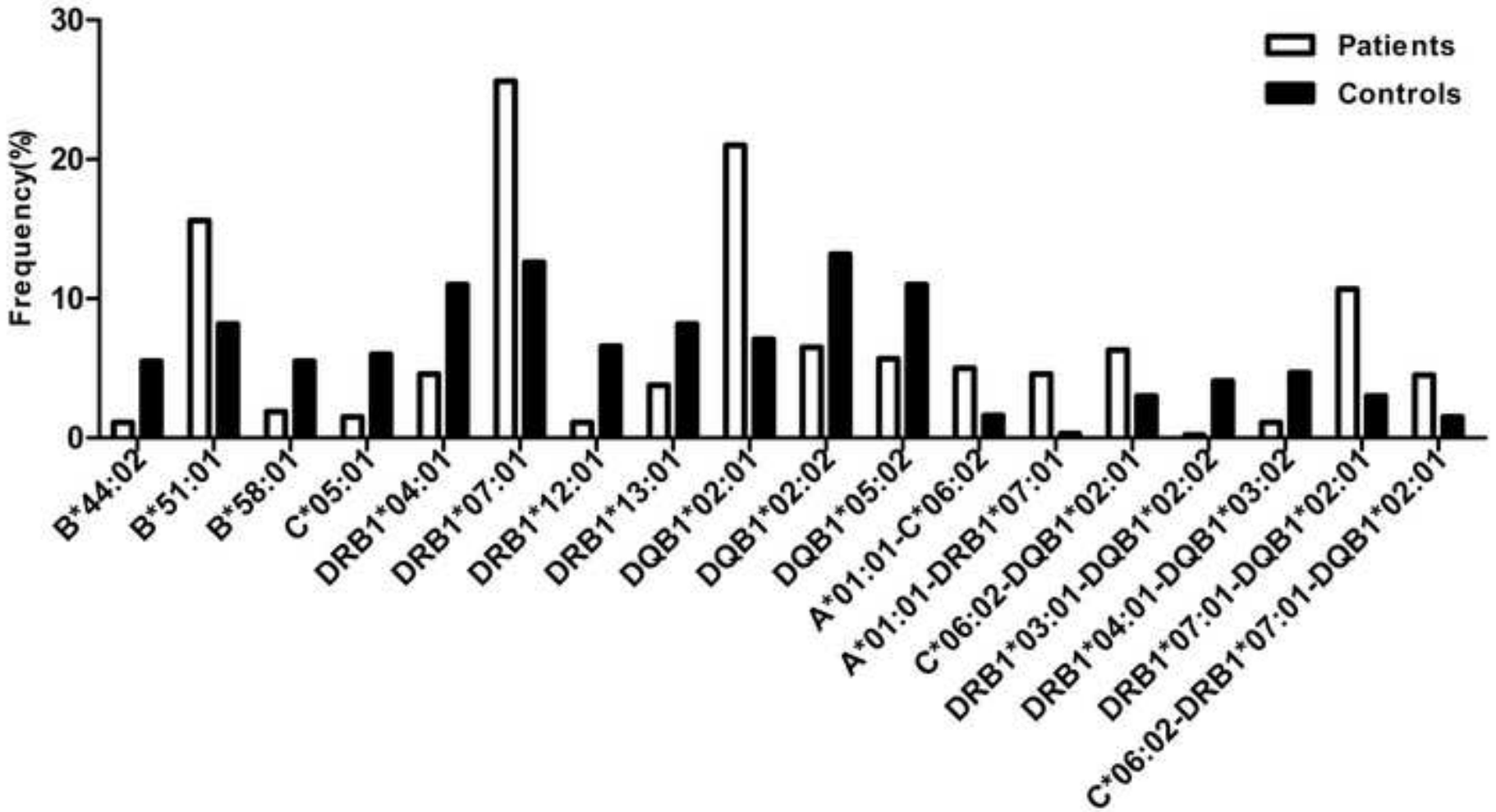

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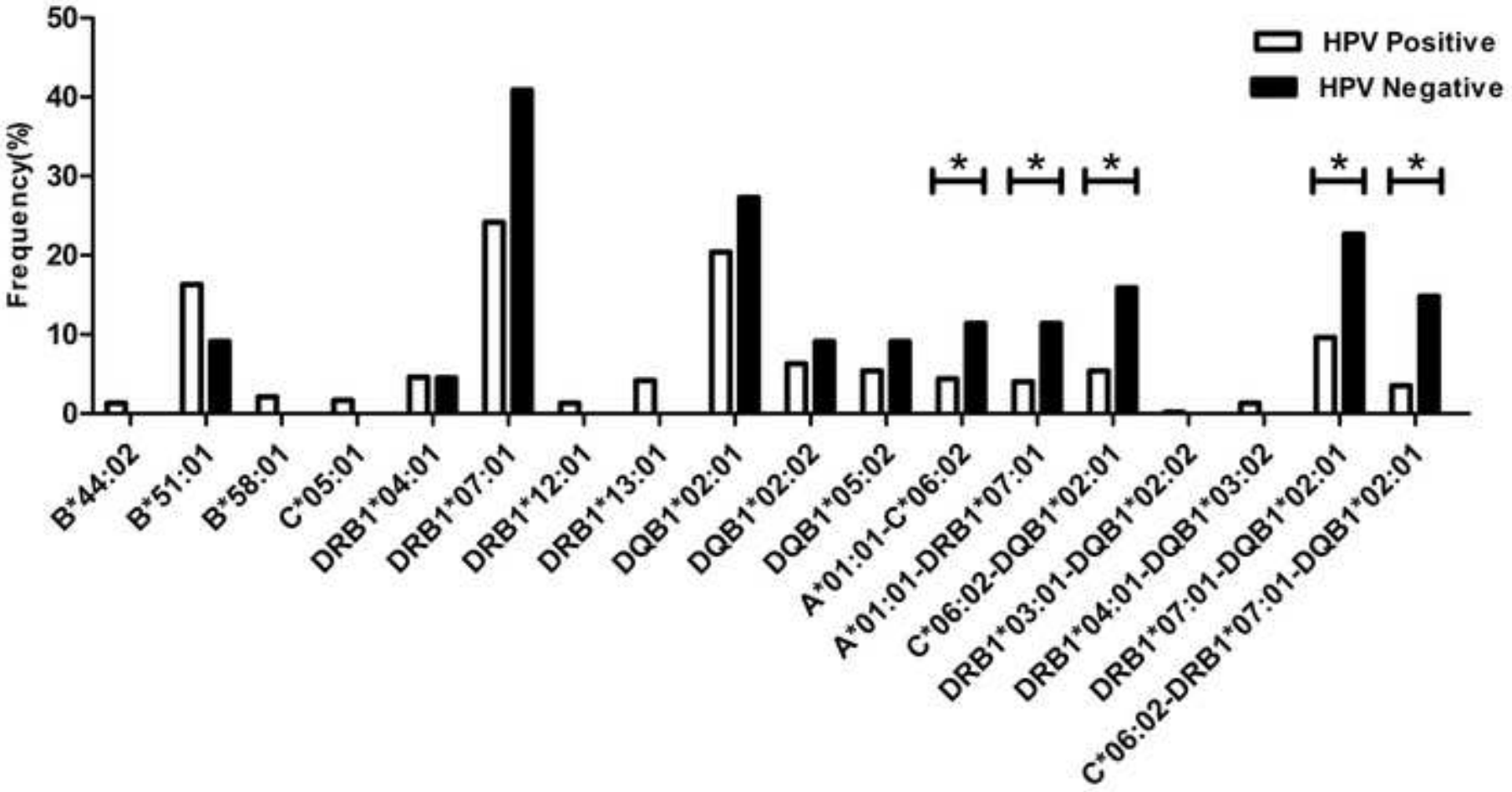

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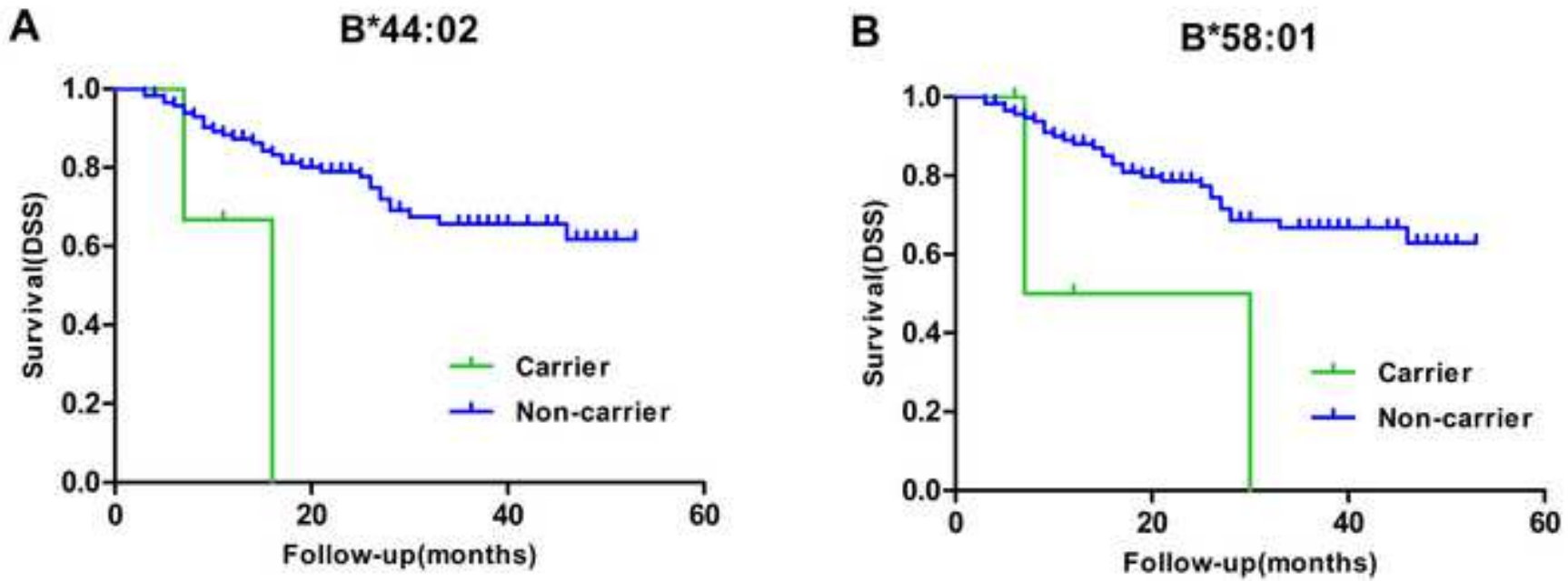

Dear Editor：

We would like to submit the enclosed manuscript entitled "Frequency distribution of HLA alleles and haplotypes in Uyghur women with advanced squamous cell cervical cancer and relation to HPV status and clinical outcome", for the publication in Archives of gynecology and obstetrics.

In this study, we have recruited the Uyghur women with advanced squamous cell cervical cancer (SCC) as well as healthy individuals living in Xinjiang, China for controls. In this study, differential frequencies of HLA alleles and haplotypes between advanced SCC individuals and healthy individuals were observed. In addition the correlation among HLA distribution, HPV status and prognosis was analyzed.

We certify that all authors have contributed significantly for this paper and are in agreement with the content of the manuscript and approved to submit to your journal. We confirm that the content of this paper has not been published or accepted elsewhere except as a brief abstract in the proceedings of a scientific meeting. It is not being submitted to any other journal. We believe the research may be of particular interest to the readers of your journal.

We sincerely appreciate your consideration of our manuscript, and look forward to receiving comments from the reviewers.

Thank you and best regards.

Yours sincerely

Mayinuer Alifu

Corresponding author:

1)Ruozheng Wang, E-mail: wrz8526@vip.163.com

2)Tao Dong, E- mail:tao.dong@imm.ox.ac.uk

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