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Protective association of HLA-DRB1*13:02, HLA-DRB1*04:06, and HLA-DQB1*06:04 alleles with cervical cancer in a Korean population

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A B S T R A C T

Human leukocyte antigen (*HLA*) class II alleles have been previously associated with cervical cancer. However, these associations vary widely across racial and ethnic groups. Therefore, we evaluated the effect of *HLA* class II alleles on cervical cancer in a Korean population. *HLA-DRB1, HLA-DQB1*, and *HLA-DQA1* alleles were analyzed in 457 cervical cancer patients and compared to those of 926 control subjects. The odds ratio (OR) of each allele between the patients and controls was calculated using the logistic regression model. Patients, had significantly lower frequencies of *HLA-DRB1* and *HLA-DQB1* alleles than control subjects: *HLA-DRB1*13:02:01* (4.4% vs 8.8%; OR 0.48, 95% confidence interval (CI) 0.27–0.84; p = 0.001), *HLA-DRB1*04:06* (2.1% vs 4.7%; OR 0.44, 95% CI 0.20–0.97; p = 0.033), and *HLA-DQB1*06:04:01* (2.3% vs 5.0%; OR 0.46, 95% CI 0.22–0.94; p = 0.021). No significant association was observed for *HLA-DQA1*. Protective associations between *HLA-DRB1*13:02*, *HLA-DRB1*04:06*, and *HLA-DQB1*06:04* alleles and cervical cancer were found in the Korean population

1. Introduction

Cervical cancer is the second most commonly occurring cancer in women worldwide [1], with human papilloma virus (HPV)-mediated infections being a major known risk factor [2]. Although infection and colonization of the cervical epithelium by HPVs initiates the development of cervical cancer, only a fraction of these infected women develop premalignant lesions, suggesting the involvement of additional co-factors in the cervical carcinogenesis process [3]. Genetic variation among several mediators of the immune response has been found to be an important determinant of neoplastic susceptibility [4], especially, in cases of HPV-induced epithelial transformations [5].

Genes encoding human leukocyte antigen (HLA) class I and II are highly polymorphic. Major histocompatibility complex (MHC) class I and II molecules expressed on the surface of antigen-presenting cells bind to, and present, the HPV peptide antigens to the cytotoxic T-cells (CD8+) and helper T-cells (CD4+) via specific receptors, respectively [6]. The genetic variability in the *HLA* may affect the binding of HPV peptides, and thus, influence the immune response to HPV infections.

Although several studies [7–10] have reported the protective and susceptible effects of certain *HLA* class II alleles on cervical cancer development or their involvement in genetic susceptibility to cervical cancer, these associations vary across different human populations. While *HLA-DRB1*15* has been associated with significantly higher odds ratio (OR) of cervical cancer in populations in the USA [9,10], the Netherlands [11], Sweden [7], Brazil [12], and India [13], *HLA-DRB1*13* has been associated with protective association in populations in the USA [9,10], the Netherlands [11], Brazil [12], Japan [3], and India [13] [Supplemental Table]. South Korea has a high incidence rate of cervical cancer, and the Korean population is genetically homogenous [14]. The investigation of *HLA* class II genes and cervical cancer in a genetically homogenous population will allow us to determine a more specific association. To date, no studies have been conducted on the correlation between *HLA* class II variability and cervical cancer in a Korean population. Our study is the first report on the analysis of *HLA* class II alleles, *HLA-DRB1*, *HLA-DQB1*, and *HLA-DQA1*, in Korean cervical cancer patients.

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2. Materials and methods

2.1. Study population

A total of 568 cancer patients and 1000 control subjects were recruited from the National Cancer Center, Korea, between August 2002 and August 2011. All the selected patients had been diagnosed with locally advanced cervical cancer of FIGO stage IB1-IVB, and were treated with primary radiotherapy, with or without chemotherapy. From this initially recruited pool, subjects with history of any other cancer type were excluded (111 patients and 74 control subjects). Finally, 457 subjects with cervical cancer and 926 control subjects were analyzed. All the blood samples used for genotyping were collected prior to any therapy. This study protocol has been approved by the Institutional review board of the National Cancer Center, Korea (IRB no. NCCCTS10412). All experiments were performed in accordance with the relevant guidelines and regulations. All subjects provided their written informed consent.

2.2. HLA class II genotyping

Genomic DNA was extracted from blood leukocytes and then amplified by polymerase chain reaction (PCR) to obtain gene fragments (exons) encoding *HLA* class II. Oligonucleotides flanking specific regions of the exons *HLA-DRB1* (exon 2 and 3), *HLA-DQB1* (exon 2 and 3), and *HLA-DQA1* (exon 2) were used for PCR. PCR was performed separately for each exon of these different genes using a conventional method with sequence-based typing (SBT) primers and Taq DNA polymerase mediated amplification (Applied Biosystems, Foster City, CA). PCR products were purified by ExoSAP-IT (USB, Cleveland, Ohio). Subsequently, the purified PCR products were subjected to a second round of PCR using Big Dye Mix (Applied Biosystems). The amplified fragments were directly sequenced in both forward and reverse directions by fluorescent capillary electrophoresis using POP-6 polymer in an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems). The *HLA* sequences were compared with reference sequences by high-resolution *HLA* typing using Match Tools software, MTNavigator software (Applied Biosystems) and Assign SBT software (Conexio-Genomics, Fremantle, Australia).

2.3. Haplotype analyses

The frequencies of *HLA-DRB1*, *HLA-DQB1*, and *HLA-DQA1* were analyzed. The numbers of analyzed *HLA* alleles were 38, 15, and 9 (for *HLA-DRB1*, *HLA-DQB1*, *HLA-DQA1* respectively). The alleles with frequencies of < 5 (12 alleles for *HLA-DRB1*) were excluded from further analyses. Ultimately 26, 15, and 9 (for *HLA-DRB1*, *HLA-DQB1*, *HLA-DQA1* respectively) types of alleles were used in the analysis. We compared haplotype frequencies between two groups using a likelihood-ratio statistic [15]. There are 115 unique haplotypes composed of three loci (*HLA-DRB1*, *HLA-DQB1*, and *HLA-DQA1*). After excluding haplotypes with estimated counts of less than five, 22 haplotypes were finally confirmed.

2.4. Statistical analysis

The demographic characteristics of study population were summarized as the mean ± standard deviation and frequency (percentage). The differences between two groups were tested using the Student *t*-test or Pearson's Chi-square test, as appropriate. Allele frequencies were summarized as counts and percentages. To investigate the risk association between patient cases and controls for each allele, the odds ratio (OR) was calculated using the logistic regression model. The Bonferroni correction was applied to determine whether there was a statistical significance, at a family-wise error rate of 0.05. The lower and upper CIs for OR were calculated after Bonferroni correction. The most frequent haplotype was chosen as the baseline. The generalized linear model (GLM) was performed with reference to the baseline haplotype to investigate the association between haplotypes and the patient-control status. The results were summarized as OR, 95% confidence interval (CI) and p-values from GLM. All statistical analyses were performed using R (version 3.3.0) software.

Table 1
Demographic characteristics for cervical cancer patients and control.

Characteristics		Control (n = 926)	Cervical cancer (n = 457)	*P-value*
Age (mean ± standard deviation)		48.52 ± 10.76	55.90 ± 14.30	< 0.0001
Age (n, %)				
	≤ 40	246 (26.6%)	72 (15.8%)	< 0.0001
	> 40	680 (73.4%)	385 (84.2%)	
Smoking status (n, %)				
	Present smoker	54 (5.8%)	48 (10.5%)	< 0.0001
	Ex-smoker	19 (2.1%)	36 (7.9%)	
	Non-smoker	790 (85.3%)	372 (81.4%)	
	Unknown	63 (6.8%)	1 (0.2%)	
Histology (n, %)				
	Squamous cell carcinoma		393 (86.0%)	
	Adeno carcinomas		50 (10.9%)	
	Unknown		14 (3.1%)	
Cervix stage (n, %)				
	I, IA1, IA2, IB1, IB2, IIA		153 (33.5%)	
	IIB, III, IIIA, IIIB, IVA		251 (54.9%)	
	IVB		37 (8.1%)	
	Unknown		16 (3.5%)	
HPV type (n, %)				
	Negative		22 (4.8%)	
	16 type		202 (44.2%)	
	18 type		43 (9.4%)	
	Other types		93 (20.4%)	
	Unknown		97 (21.2%)	

HPV*, human papillomavirus.

3. Results

The demographic characteristics for 457 subjects with cervical cancer and 926 control subjects are shown in Table 1. The mean value for the age category was statistically higher in the cervical cancer group (55.90 ± 14.30) than in the normal group (48.52 ± 10.76). In both groups, most subjects were non-smokers, but there were significantly more current and ex-smokers found in the cervical cancer group. Among the cervical cancer patients, 393 (86.0%) patients had squamous cell carcinoma, whereas 288 (63.0%) patients were stage IIB or higher. The number of patients diagnosed with stage IVB, in which metastasis was present, was 37. This would not affect the overall result. HPV type 18 was present in 43 (9.4%) patients of the cervical cancer group.

The allele frequencies and ORs of *HLA-DRB1* are shown in Table 2. The allele frequency of the control group was compared with previously published data [16] and no statistically significant difference was found. The most frequent allele was *HLA-DRB1*09:01:02*, with a frequency of 11.7% in the patient group and 10.6% in the control group. The second-most frequent allele was *HLA-DRB1*15:01:01*, with a frequency of 9.9% in the patient group and 8.1% in the control group. Both alleles, *HLA-DRB1*09:01:02* and *HLA-DRB1*15:01:01*, showed no significant difference between the patient and control groups. Strikingly, we observed a significantly lower frequency of alleles *HLA-*

Table 2
Allele frequency of DRB1.

DRB1	Control	Case	OR	Corrected 95% CI		p-value*
	Count (%)	Count (%)		LCI	UCI	
01:01:01	114 (6.18)	50 (5.51)	0.885	0.515	1.522	1.0000
03:01	27 (1.46)	15 (1.65)	1.132	0.413	3.097	1.0000
04:01:01	11 (0.60)	11 (1.21)	2.046	0.542	7.725	1.0000
04:03:01	67 (3.63)	36 (3.96)	1.096	0.570	2.108	1.0000
04:04:01	23 (1.25)	10 (1.10)	0.883	0.271	2.877	1.0000
04:05	147 (7.96)	69 (7.60)	0.951	0.593	1.523	1.0000
04:06	**86 (4.66)**	**19 (2.09)**	**0.437**	**0.197**	**0.970**	**0.0333**
04:07:01	11 (0.60)	8 (0.88)	1.483	0.349	6.301	1.0000
04:10	24 (1.30)	16 (1.76)	1.362	0.496	3.735	1.0000
07:01	131 (7.10)	74 (8.15)	1.162	0.726	1.858	1.0000
08:02:01	42 (2.28)	26 (2.86)	1.266	0.578	2.774	1.0000
08:03:02	129 (6.99)	75 (8.26)	1.198	0.750	1.916	1.0000
09:01:02	196 (10.62)	106 (11.67)	1.113	0.748	1.655	1.0000
10:01:01	29 (1.57)	19 (2.09)	1.339	0.531	3.375	1.0000
11:01	73 (3.95)	56 (6.17)	1.596	0.906	2.811	0.2695
12:01:01	90 (4.88)	32 (3.52)	0.713	0.372	1.367	1.0000
12:02:01	54 (2.93)	23 (2.53)	0.862	0.394	1.887	1.0000
13:01:01	39 (2.11)	13 (1.43)	0.673	0.247	1.832	1.0000
13:02:01	**163 (8.83)**	**40 (4.41)**	**0.476**	**0.271**	**0.835**	**0.0011**
14:01:01	57 (3.09)	30 (3.30)	1.072	0.527	2.184	1.0000
14:03:01	19 (1.03)	9 (0.99)	0.963	0.273	3.399	1.0000
14:05:01	51 (2.76)	22 (2.42)	0.874	0.392	1.948	1.0000
14:06:01	18 (0.98)	6 (0.66)	0.676	0.156	2.931	1.0000
15:01:01	149 (8.07)	90 (9.91)	1.253	0.811	1.935	1.0000
15:02:01	54 (2.93)	35 (3.85)	1.330	0.671	2.640	1.0000
16:02:01	21 (1.14)	10 (1.10)	0.968	0.292	3.208	1.0000

OR;Odds ratio, Corrected 95% CI: (1–0.05/26)% confidence interval.
LCI; lower confidence interval, UCI; upper confidence interval.
p-value*: Bonferroni-corrected p-value.

*DRB1*13:02:01* (4.4%-patient group vs 8.8% control group; OR 0.48, 95% CI 0.27–0.84; p = 0.001) and *HLA-DRB1*04:06* (2.1% patient group vs 4.7% control group; OR 0.44, 95% CI 0.20–0.97; p = 0.033).

The allele frequencies and odd ratios of *HLA-DQB1* and *HLA-DQA1* are shown in Tables 3 and 4. The cervical cancer patient group showed a lower frequency of the allele *HLA-DQB1*06:04:01* (2.3% patient group vs 5.0% control group; OR 0.46, 95% CI 0.22–0.94; p = 0.021), whereas no significant association was observed for *HLA-DQA1*.

Allele frequencies of over 1% were observed in at least one group of 22 haplotypes (Table 5). The allele *HLA-DRB1*09:01:02-DQB1*03:03:02-DQA1*03:01* was the most frequent, with frequencies of 11.4% in the patient group and 10.4% in the control group. Frequencies of alleles *HLA-DRB1*13:02:01-DQB1*06:04:01-DQA1*01:02* (2.2% patient group vs 5.0% control group; OR 0.40, 95% CI 0.23–0.70; p < 0.001), *HLA-DRB1*04:06-DQB1*03:02:01-DQA1*03:01* (2.1% patient group vs 4.5% control group; OR 0.42, 95% CI 0.24–0.73; p = 0.002), and *HLA-DRB1*13:02:01-DQB1*06:09-DQA1*01:02* (1.9% patient group vs 3.9% control group; OR 0.45, 95% CI 0.24–0.82; p = 0.005) were significantly lower in the patient group than in the control group.

Table 3
Allele frequency of DQB1.

DQB1	Control	Case	OR	Corrected 95% CI		p-value*
	Count (%)	Count (%)		LCI	UCI	
02:01:01	47 (2.55)	15 (1.66)	0.642	0.267	1.546	1.0000
02:02:01	100 (5.43)	66 (7.28)	1.367	0.845	2.213	0.8495
03:01:01	249 (13.53)	137 (15.12)	1.138	0.812	1.596	1.0000
03:02:01	208 (11.30)	91 (10.04)	0.876	0.593	1.294	1.0000
03:03:02	237 (12.88)	126 (13.91)	1.093	0.771	1.547	1.0000
04:01:01	146 (7.93)	75 (8.28)	1.047	0.678	1.618	1.0000
04:02:01	63 (3.42)	29 (3.20)	0.933	0.477	1.822	1.0000
05:01:01	152 (8.26)	74 (8.17)	0.988	0.640	1.525	1.0000
05:02:01	64 (3.48)	28 (3.09)	0.885	0.450	1.740	1.0000
05:03:01	71 (3.86)	36 (3.97)	1.031	0.559	1.903	1.0000
06:01:01	161 (8.75)	100 (11.04)	1.294	0.872	1.919	0.8271
06:02:01	141 (7.66)	80 (8.83)	1.167	0.760	1.793	1.0000
06:03:01	39 (2.12)	10 (1.10)	0.515	0.181	1.469	0.9483
06:04:01	**91 (4.95)**	**21 (2.32)**	**0.456**	**0.222**	**0.938**	**0.0208**
06:09	71 (3.86)	18 (1.99)	0.505	0.231	1.106	0.1580

OR; Odds ratio, Corrected 95% CI: (1–0.05/15)% confidence interval.
LCI; lower confidence interval, UCI; upper confidence interval.
p-value*: Bonferroni-corrected p-value.
Bold value is the type that has the statistical significance (p = 0.0208) in protective effect of cervical cancer.

Table 4
Allele frequency of DQA1.

DQA1	Control	Case	Odds	Corrected 95% CI		p-value*
	Count (%)	Count (%)	Ratio	LCI	UCI	
01:01	270 (14.75)	130 (14.29)	0.963	0.699	1.326	1.0000
01:02	327 (17.87)	130 (14.29)	0.766	0.560	1.047	0.1623
01:03	202 (11.04)	113 (12.42)	1.143	0.808	1.617	1.0000
02:01	113 (6.17)	76 (8.35)	1.385	0.903	2.124	0.3137
03:01	620 (33.88)	302 (33.19)	0.969	0.764	1.230	1.0000
04:01	32 (1.75)	17 (1.87)	1.070	0.462	2.477	1.0000
05:01:01	172 (9.40)	112 (12.31)	1.353	0.946	1.934	0.1707
05:01:02	23 (1.26)	3 (0.33)	0.260	0.047	1.431	0.2564
06:01:01	71 (3.88)	27 (2.97)	0.758	0.401	1.433	1.0000

Corrected 95% CI: (1–0.05/9)% confidence interval.
LCI; lower confidence interval, UCI; upper confidence interval.
p-value*: Bonferroni-corrected p-value.
Bold value is the type that has the statistical significance (p = 0.0208) in protective effect of cervical cancer.

4. Discussion

This study provides evidence for *HLA-DRB1*13:02:01* and *HLA-DRB1* 04:06* as having a protective association with cervical cancer development. Other studies have previously revealed a negative correlation between *HLA-DRB1*13* and cervical cancer in Hispanic [9] and Dutch [11] populations. Both findings, observed in HPV positive cases, suggest a protective effect of this antigen against HPV-associated cervical cancer. *HLA-DRB1*13:02:01* was previously found with lesser frequency among cervical cancer patients in Chinese [8,17], French [18], Brazilian [12], and other [7,10] populations. A prospective cohort study [19], which investigated the relationship between *HLA-DRB1*13* alleles and cytological regression in French women with CIN1 (cervical intraepithelial neoplasia grade 1), had previously suggested the protective effects of this allele, albeit with limitations due to the small sample size of the study (n = 86).

Several carcinogenic steps are required in the development of cervical cancer: HPV infection, HPV persistence (development of low-grade cervical precursor lesion), progression of a persisting infection to cervical precancer, and finally, invasion through the epithelial basement membrane [2,20]. The exact step at which *HLA-DRB1*13* alleles exert their protective effect is not yet fully understood. However, several prospective studies [21–23] of HPV infections have shown that *HLA-DRB1*13* alleles do not play a protective role against the acquisition and persistence of viral infections, but rather, play a role during the progression to cervical precancer stage. The possible role of *HLA-DRB1*13:02* allele in the progression to cervical cancer may contribute to the recognition of viral antigens such as the E7 protein, which increases during progression to cervical precancer [24,25]. In future, the identification of specific viral epitopes presented by the *HLA-DRB1*13:02* alleles will enhance our understanding of this effect.

In another study, *HLA-DRB1*13:02* was found to have protective association with cervical cancer patients in Japanese population [3]. The authors conducted cytology and colposcopy tests for 454 women with low-grade squamous intraepithelial lesion, every 3–4 months, to analyze the cumulative risk of CIN3 in the following 10 years with

Table 5
Haplotype frequency.

DRB1	DQB1	DQA1	Control	Case	p-value	OR	95% CI	
			%	%			LCI	UCI
13:02:01	**06:04:01**	**01:02**	**4.99%**	**2.21%**	**0.0004**	**0.398**	**0.228**	**0.697**
04:06	**03:02:01**	**03:01**	**4.49%**	**2.10%**	**0.0020**	**0.415**	**0.236**	**0.731**
13:02:01	**06:09**	**01:02**	**3.89%**	**1.88%**	**0.0048**	**0.448**	**0.244**	**0.824**
13:01:01	06:03:01	01:03	1.97%	1.10%	0.0935	0.559	0.253	1.235
12:01:01	03:03:02	03:01	1.48%	0.88%	0.1557	0.490	0.210	1.143
12:02:01	03:01:01	06:01:01	2.96%	2.43%	0.4256	0.785	0.447	1.377
01:01:01	05:01:01	01:01	6.14%	5.52%	0.5180	0.823	0.540	1.255
14:05:01	05:03:01	01:01	2.63%	2.32%	0.6236	0.829	0.463	1.485
14:01:01	05:02:01	01:01	2.24%	1.99%	0.6726	0.812	0.440	1.497
04:05	04:01:01	03:01	7.95%	7.51%	0.6878	0.855	0.584	1.252
16:02:01	05:02:01	01:02	1.15%	0.99%	0.7078	0.793	0.340	1.849
04:10	04:02:01	03:01	1.26%	1.21%	0.9169	0.913	0.423	1.967
12:01:01	03:01:01	05:01:01	2.32%	2.21%	0.9352	0.906	0.498	1.649
04:03:01	03:02:01	03:01	3.56%	3.86%	0.6879	1.039	0.632	1.709
09:01:02	**03:03:02**	**03:01**	**10.41%**	**11.37%**	**0.4421**	**Base**		
10:01:01	05:01:01	01:01	1.59%	2.10%	0.3482	1.235	0.645	2.364
03:01	02:01:01	05:01:01	1.10%	1.55%	0.3164	1.307	0.619	2.758
15:01:01	06:02:01	01:02	7.29%	8.50%	0.2597	1.017	0.691	1.497
08:03:02	06:01:01	01:03	6.25%	7.40%	0.2451	1.093	0.724	1.648
15:02:01	06:01:01	01:03	2.25%	3.42%	0.0677	1.401	0.817	2.403
07:01	02:02:01	02:01	4.50%	7.06%	0.0044	1.464	0.961	2.229
11:01	03:01:01	05:01:01	3.31%	5.85%	0.0016	1.515	0.954	2.404

OR; Odds ratio, Corrected 95% CI: (1–0.05/15)% confidence interval.
LCI; lower confidence interval, UCI; upper confidence interval.
p-value: Bonferroni-corrected p-value.
Bold value is the type that has the statistical significance (p = 0.0208) in protective effect of cervical cancer.

respect to class II alleles [21]. They found that the cumulative risk of CIN3 diagnosis, within 10 years, was significantly lower among *HLA-DRB1*13:02* positive women (3.2% for *HLA-DRB1*13:02* positive vs 23.7% for *HLA-DRB1*13:02* negative; p = 0.03); this result also supports our theory that *HLA-DRB1*13:02* has a protective effect at the cancer progression level.

In this study, *HLA-DQB1*06:04:01* also showed protective association with cervical cancer. However, the haplotype analysis revealed that most of the *HLA-DQB1*06:04:01* co-existed with *HLA-DRB1*13:02:01*, making it difficult to delineate whether the observed protective effect was due to the *HLA-DRB1* allele. A meta-analysis in patients from Europe and Asia has previously revealed the protective association of *HLA-DQB1*02*, *HLA-DQB1*03*, and *HLA-DQB1*06:03* alleles with cervical cancer [26]. A study that analyzed the amino acid profile for specific *HLA-DQB1** alleles in cervical cancer patients in Southern India [13] revealed that amino acid substitutions at β9 (P6 pockets) and β37 (P9 pocket) positions of various *HLA-DQB1** alleles showed a higher occurrence of 'tyrosine' residue replacement in cervical cancer patients. This effect was statistically significant at a genotypic level; tyrosine/non-tyrosine combinations at both β9 (P6 pockets) and β37 (P9 pocket) were associated with an increased OR of cervical cancer, when compared to tyrosine/tyrosine and non-tyrosine/non-tyrosine combinations. Different amino acid residues in the peptide may alter the shape and binding affinity within the HLA groove and thus affect the immune response.

In our study, *HLA- DRB1*04:06* was associated with protective effect on cervical cancer. Similar results were obtained in a study of a Caucasian population in USA [10], however, studies conducted in India [24] and a Hispanic population in USA [9] found a converse association between *HLA-DRB1*04* and protective effect on cervical cancer. The reported phenotype frequency of *HLA-DRB1*04:06* is 10.6–10.9% in South Korean population, whereas in USA Caucasian population, it is reported to be 0.2% [27]. The opposite effect may be explained by relatively large difference of allele frequencies among different ethnic groups.

Chronic inflammation caused by HPV infection precedes tumor development. However, whether the immune response of HLA is directed against the infectious agent or tumor antigens is not well known [28]. A potential association between *HLA* polymorphism and serum cytokine level was reported [29] with certain *HLA* types being related to statistically significant levels of IL-10, which showed a protective effect. *HLA* genes that are important for both immune recognition and clearance of infectious agent would result in different susceptibility to HPV and tumor progression [10,30,31]. Optimal peptide presentation by MHC class I and II would activate cytotoxic and helper T-cells, respectively. Changes in T-cell response may allow escape from immune surveillance and thus, result in tolerance to HPV peptides [32].

There are a few limitations in this study, as discussed below. Firstly, there is no correlation study between the *HLA* class II alleles and the specific HPV types. The biochemical and biological differences between E7 oncoproteins of the high- and low-risk HPV types are determined by their amino-terminal sequences. This difference can affect the affinity for binding to and transformation of certain cells, and affect the oncogene [33]. Therefore, it is plausible that different structures and epitopes of various HPV types could influence the recognition by HLA molecules.

Second, this study does not examine *HLA* class I alleles. *HLA* class I alleles and their effect on cervical cancer has been previously examined [17]. While one genotype or haplotype is not likely to individually cause the disease, but rather interplay between host's genetic factors, environmental factors, and HPV-related factors. *HLA* class I alleles as well as other factors such as single nucleotide polymorphisms (SNPs) in cytokine genes and receptor/*KIR* genes are of equal significance as *HLA* class II alleles in immune response.

Previous studies have demonstrated the association of different *HLA* alleles with cervical cancer development. However, the use of multiple genotyping methods can result in different levels of resolution of *HLA* alleles. Our study, based on sequence typing data, produced four digits form, nevertheless, there are still some challenges when comparing data from several studies with different *HLA* typing resolution. Further high-resolution analyses will result in more accurate and consistent conclusions about the role of *HLA* alleles in cervical cancer.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humimm.2018.10.013.

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