Cancer Immunol Immunother (2015) 64:1151–1157
DOI 10.1007/s00262-015-1721-5

ORIGINAL ARTICLE

Genetic susceptibility to cervical squamous cell carcinoma is associated with HLA-DPB1 polymorphisms in Taiwanese women

Yuh-Cheng Yang[1,4] · Tzu-Yang Chang[3] · Tze-Chien Chen[1] · Wen-Shan Lin[3] · Shih-Chuan Chang[3] · Yann-Jinn Lee[2,3,5,6]

Received: 26 November 2014 / Accepted: 21 May 2015 / Published online: 2 June 2015

Abstract Cervical cancer is a multifactorial disease, and increasing evidence suggests that host immunogenetic background may contribute to its pathogenesis. Genetic variations in human leukocyte antigen (HLA) genes may alter the efficiency of immune response to human papillomavirus (HPV) antigens and have been implicated in the risk of cervical cancer. We investigated whether polymorphisms in the HLA-DPB1 gene were associated with cervical cancer risk in a Taiwanese population. HLA-DPB1 alleles and +550 G/A polymorphism were genotyped in a case–control study of 473 women with cervical squamous cell carcinoma (CSCC) and 676 healthy controls. The presence and genotypes of HPV in CSCC were determined. We found that the DPB1*05:01 and +550 A alleles were associated with decreased and increased risk of CSCC, respectively [odds ratio (OR) = 0.72, $P_c = 0.001$; OR = 1.25, $P_c = 0.03$]. In subgroup analysis based on HPV type 16 positivity, significant associations were shown in the DPB1*05:01 and *13:01 alleles (OR = 0.65, $P_c = 0.0007$; OR = 1.83, $P_c = 0.004$). Furthermore, the DPB1*05:01-G and *13:01-G haplotypes conferred decreased and increased risk of both CSCC and HPV-16 positive CSCC women, respectively (OR = 0.72, $P_c = 0.0009$; OR = 0.63, $P_c = 0.0004$ for DPB1*05:01-G; OR = 1.55, $P_c = 0.03$; OR = 1.84, $P_c = 0.004$ for DPB1*13:01-G). A risk haplotype DPB1*02:01-A was also observed in the HPV-16 positive CSCC women (OR = 1.51, $P_c = 0.05$). These findings suggest that HLA-DPB1 gene is involved in the CSCC development.

Keywords Cervical cancer · HPV · Immunity · HLA-DPB1 · Polymorphism

Abbreviations

CI	Confidence interval
CSCC	Cervical squamous cell carcinoma
HLA	Human leukocyte antigen
HPV	Human papillomavirus
LD	Linkage disequilibrium
OR	Odds ratio
PCR	Polymerase chain reaction

Introduction

Cervical cancer is the third most commonly diagnosed cancer and the fourth leading cause of cancer death in women worldwide [1]. It is also a grave health problem in Taiwan,

This study was presented in part as abstract in poster at the 63rd Annual Meeting of American Society of Human Genetics, Boston, USA, October 22–26, 2013. Program Guide, p 246 (abstract 3265T).

Yuh-Cheng Yang and Tzu-Yang Chang have contributed equally to this work.

✉ Yann-Jinn Lee
yannlee@mmh.org.tw; kylechang@mmh.org.tw

1 Department of Gynecology and Obstetrics, Mackay Memorial Hospital, Taipei City, Taiwan

2 Department of Pediatrics, Mackay Memorial Hospital, Taipei City, Taiwan

3 Department of Medical Research, Mackay Memorial Hospital, New Taipei City, Taiwan

4 Department of Gynecology and Obstetrics, Taipei Medical University, Taipei City, Taiwan

5 Department of Pediatrics, Taipei Medical University, Taipei City, Taiwan

6 Institute of Biomedical Sciences and Department of Medicine, Mackay Medical College, New Taipei City, Taiwan

with 2700 women developing this disease each year and second in incidence only to breast cancer [2]. Human papillomavirus (HPV) has been widely accepted as an important etiological agent of cervical carcinogenesis [3]. However, only a small portion of HPV-infected women progress to cervical cancer during their lifetime, suggesting that other factors may contribute to the progression of the disease. Along with environmental and lifestyle factors, familial aggregation studies indicate that host genetic variation is likely to involve in cervical cancer pathogenesis [4].

Human leukocyte antigen (HLA) genes are highly polymorphic and encode HLA molecules that are essential for the presentation of viral peptides to the immune system, including HPV. The large number of polymorphisms of the HLA alleles leads to variations in the antigen-recognition site, which may confer susceptibility or resistance to HPV infection and the consequentially neoplastic progression or regression. Due to the pivotal role of HLA molecules in the immune system, several studies have been performed to investigate the association between specific HLA alleles and cervical cancer. In a review article, Hildesheim and Wang [5] reported that HLA-DRB1*15:01, DQB1*03 alleles, and DRB1*15:01-DQB1*06:02 haplotype were positively associated with cervical cancer among certain ethnic groups, whereas DRB1*13 alleles and DRB1*13:01-DQB1*06:03 haplotype were negatively associated with it. Other studies have shown that HLA-A*03:01, *11:04, B*44:02, and Cw*05:01 alleles conferred susceptibility to cervical cancer, but a protective effect against the disease was found in HLA-A*24:02, B*15:01, and Cw*02:02 alleles [6–8]. However, only a few studies have explored the association between HLA-DPB1 and cervical cancer [9–11] and no such data on Taiwanese population have been reported.

In this study, we investigated whether HLA-DPB1 alleles are associated with cervical cancer risk in a hospital-based case–control study of 473 cervical squamous cell carcinoma (CSCC) patients and 676 sex- and age-matched healthy controls. A recently discovered SNP, +550 G/A (dbSNP ID: rs 9277535), located within the 3′ UTR of HLA-DPB1 gene, also has been analyzed because of its putative role in the regulation of HLA-DPB1 mRNA expression [12].

Patients and methods

Study population

A hospital-based case–control study was conducted at the Mackay Memorial Hospital. Four hundred and seventy-three Taiwanese women with pathologically proven CSCC (mean ± SD age at diagnosis: 53.7 ± 12.1 years) were included in the present study. Controls were 676 women (mean ± SD age at sampling: 54.5 ± 8.4 years) selected randomly from a cervical cancer screening program. Exclusion criteria included abnormal Pap result, history of cervical neoplasia, skin or genital warts, immunocompromised conditions, other cancers, and previous operations on the uterine cervix. All the control subjects were frequency matched to the cases by age. After obtaining informed consent, surgical resections or cervical scrapes were collected from all genetically unrelated study participants. The study was approved by the ethics committee and adhered to the tenets of the 1964 Declaration of Helsinki.

DNA isolation

DNA was isolated from formalin-fixed and paraffin-embedded tissue specimens of CSCC patients by use of the Qiagen DNeasy Tissue Kit (Qiagen, Valencia, CA) according to the manufacturer's protocol. Qiagen DNA extraction kit was used to isolate DNA from cervical scrapes of controls.

HPV typing

HPV detection and genotyping were performed by polymerase chain reaction (PCR)-based amplification of a fragment of approximately 192 bp in the L1 region of the HPV genome with a pair of degenerate primers, GP6+/MY11 [13, 14]. The HPV genotype was then determined by sequencing the PCR product on an automated sequencer (ABI 377, Applied Biosystems, Foster City, CA). Since stratifications based on HPV types in controls were not performed in this study, no HPV DNA testing was done for the control subjects.

HLA-DPB1 genotyping

HLA-DPB1 alleles were determined using high-resolution sequence-based typing protocol with some modifications from a previous study [15]. This involved a high-resolution HLA typing method using PCR amplification of genomic DNA, followed by direct DNA sequencing. Briefly, PCR using locus-specific primer sets was performed to amplify exon 2 of HLA-DPB1 gene. The presence of PCR product was checked by 2 % agarose gel electrophoresis. The amplicons were then purified and sequenced using the ABI BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA) in an ABI 3730 DNA analyzer. Sequencing was performed in both forward and reverse directions and analyzed using Assign-SBT™ 3.5+ software (Conexio Genomics, Fremantle, Australia). Ambiguous alleles were assigned according to common alleles (allele frequency >0.01) found in the Taiwanese and southern Chinese populations [16, 17] or to the allele with

Table 1 Distribution of HLA-DPB1 alleles in controls, women with CSCC and those with HPV-16 positive CSCC

DPB1 Alleles	Controls ($2N = 1352$)	CSCC ($2N = 946$)	HPV-16 positive CSCC ($2N = 450$)	CSCC		HPV-16 positive CSCC	
	No. (%)	No. (%)	No. (%)	OR (95 % CI)	P_c value	OR (95 % CI)	P_c value
02:01	181 (13.4)	145 (15.3)	78 (17.3)	1.17 (0.92–1.48)	0.81	1.36 (1.02–1.81)	0.27
02:02	97 (7.2)	60 (6.4)	26 (5.8)	0.88 (0.63–1.22)	0.99	0.79 (0.51–1.24)	0.95
03:01	69 (5.1)	55 (5.8)	34 (7.6)	1.15 (0.81–1.65)	0.99	1.52 (0.99–2.33)	0.35
04:01	92 (6.8)	70 (7.4)	24 (5.3)	1.09 (0.79–1.51)	1.00	0.77 (0.49–1.23)	0.92
05:01	649 (48.0)	379 (40.1)	168 (37.4)	0.72 (0.61–0.86)	0.001	0.65 (0.52–0.80)	0.0007
13:01	101 (7.5)	100 (10.6)	58 (12.9)	1.46 (1.10–1.96)	0.08	1.83 (1.31–2.57)	0.004
14:01	31 (2.3)	24 (2.5)	14 (3.1)	1.11 (0.65–1.91)	1.00	1.37 (0.72–2.61)	0.96
21:01	35 (2.6)	19 (2.0)	7 (1.6)	0.77 (0.44–1.36)	0.97	0.59 (0.26–1.35)	0.85
Others[a]	97 (7.1)	94 (9.9)	41 (9.0)				

CSCC cervical squamous cell carcinoma, *HPV* human papillomavirus, *OR* odds ratio, *CI* confidence interval

P_c value = Bonferroni corrected *P* value. The correction number is 8

[a] Others indicate grouping of the remaining alleles with frequencies lower than 1.5 % in controls or patients

lowest definition number. The genotyping of the +550 G/A SNP was performed with the TaqMan assay (Applied Biosystems) according to the manufacturer's instructions.

Statistical analysis

Statistical significance of differences in frequency between each allele, genotype, and haplotype of CSCC, HPV-16 positive CSCC, and control groups was compared using Chi-square test with Yates' correction or Fisher's exact test (when the number of subjects in a cell was <5). Odds ratio (OR) and 95 % confidence interval (CI) were calculated to determine the magnitude of associations. Hardy–Weinberg equilibrium, haplotype frequencies, and linkage disequilibriums (LDs) were evaluated with PyPop 0.7.0 statistical software [18] from the International Histocompatibility Working Group based on the method described by Guo and Thompson [19]. Haplotypes with frequency <1.5 % in the cases or controls were excluded. The Bonferroni correction was used to correct *P* values when several statistical tests were being performed simultaneously on a single data set. To do a Bonferroni correction, the formula $P_c = 1 - (1 - P)^n$ was applied, where P_c is the corrected *P* value, *P* the uncorrected one, and n the number of comparisons [20]. The comparison number was 8 for HLA-DPB1 alleles and haplotypes. As for +550 G/A SNP, n is 2 for each genotype and allele for simultaneously testing genotype and allele frequencies [21]. A corrected *P* value (P_c) of <0.05 (2-tailed) was considered statistically significant. Using the Quanto Version 1.1 software (Department of Preventive Medicine, University of Southern California, CA, USA), we designed the study to have >80 % power at a 5 % significance level to determine a genotype relative risk of 1.5 conferred by HLA-DPB1 variants with an estimated prevalence of 360/100,000 [22].

Results

HPV DNA was detected in 72.7 % of the 473 tumors tested, with 65.4 % positive for HPV type 16, 10.2 % for HPV type 18, and 24.4 % for other HPV types.

The distribution of the HLA-DPB1 alleles and +550 G/A genotypes and alleles in the controls and cases is shown in Tables 1 and 2. The observed genotype frequencies for these DPB1 variants did not deviate significantly from the Hardy–Weinberg equilibrium in the controls ($P > 0.05$). A significantly increased frequency in DPB1*13:01 allele (OR = 1.46, 95 % CI 1.10–1.96) and +550 A allele (OR = 1.25, 95 % CI 1.05–1.49) and a significantly decreased frequency in DPB1*05:01 allele (OR = 0.72, 95 % CI 0.61–0.86), +550 G/G genotype (OR = 0.75, 95 % CI 0.59–0.96), and G allele (OR = 0.80, 95 % CI 0.67–0.96) were found in CSCC patients as compared with controls (Tables 1, 2). The significance was still retained in DPB1*05:01 allele ($P_c = 0.001$), +550 G/G genotype ($P_c = 0.04$), and A and G alleles ($P_c = 0.03$) after Bonferroni correction.

In order to clarify whether interactions between oncogenic HPV-16 infection and HLA-DPB1 variants would affect susceptibility or progression to CSCC, we further stratified CSCC patients by positivity of HPV type 16. The results showed that the frequencies of DPB1*13:01 allele increased significantly (OR = 1.83, 95 % CI 1.31–2.57) and the DPB1*05:01 allele decreased significantly (OR = 0.65, 95 % CI 0.52–0.80) in women with HPV-16 positive CSCC

Table 2 Genotype and allele frequencies of the HLA-DPB1 +550 G/A polymorphism in controls, women with CSCC and those with HPV-16 positive CSCC

	Controls ($N = 676$)	CSCC ($N = 473$)	HPV-16 positive CSCC ($N = 225$)	CSCC		HPV-16 positive CSCC	
	No. (%)	No. (%)	No. (%)	OR (95 % CI)	P_c value	OR (95 % CI)	P_c value
Genotype							
G/G	320 (47.3)	191 (40.4)	96 (42.7)	0.75 (0.59–0.96)	0.04	0.83 (0.61–1.12)	0.40
G/A	287 (42.5)	220 (46.5)	96 (42.7)	1.18 (0.93–1.49)	0.32	1.01 (0.74–1.37)	1.00
A/A	69 (10.2)	62 (13.1)	33 (14.6)	1.33 (0.92–1.91)	0.24	1.51 (0.97–2.36)	0.13
Allele							
G	927 (68.6)	602 (63.6)	288 (64.0)	0.80 (0.67–0.96)	0.03	0.82 (0.65–1.02)	0.14
A	425 (31.4)	344 (36.4)	162 (36.0)	1.25 (1.05–1.49)		1.23 (0.98–1.54)	

CSCC cervical squamous cell carcinoma, *HPV* human papillomavirus, *OR* odds ratio, *CI* confidence interval

P_c value = Bonferroni corrected *P* value. The correction number is 2

Table 3 Analysis of HLA-DPB1 haplotypes in controls, women with CSCC and those with HPV-16 positive CSCC

Haplotype	Controls ($2N = 1352$)	CSCC ($2N = 946$)	HPV-16 positive CSCC ($2N = 450$)	CSCC		HPV-16 positive CSCC	
	No. (%)	No. (%)	No. (%)	OR (95 % CI)	P_c value	OR (95 % CI)	P_c value
02:01-A	163 (12.1)	144 (15.2)	77 (17.1)	1.31 (1.03–1.67)	0.21	1.51 (1.12–2.02)	0.05
02:02-A	93 (6.9)	60 (6.3)	26 (5.8)	0.92 (0.66–1.28)	1.00	0.83 (0.53–1.31)	0.99
03:01-G	64 (4.7)	55 (5.8)	34 (7.6)	1.24 (0.86–1.81)	0.90	1.64 (1.07–2.53)	0.16
04:01-A	85 (6.3)	69 (7.3)	23 (5.1)	1.17 (0.84–1.63)	0.96	0.81 (0.51–1.29)	0.97
05:01-G	633 (46.8)	366 (38.7)	161 (35.8)	0.72 (0.61–0.85)	0.0009	0.63 (0.51–0.79)	0.0004
13:01-G	95 (7.0)	99 (10.5)	55 (12.2)	1.55 (1.15–2.08)	0.03	1.84 (1.30–2.62)	0.004
14:01-G	29 (2.1)	24 (2.5)	14 (3.1)	1.19 (0.69–2.05)	1.00	1.46 (0.77–2.81)	0.89
21:01-G	33 (2.4)	19 (2.0)	7 (1.6)	0.82 (0.46–1.45)	1.00	0.63 (0.28–1.44)	0.92

Haplotype inferred using PyPop 0.7.0 software, based on the HLA-DPB1 alleles and +550 G/A polymorphism

CSCC cervical squamous cell carcinoma, *HPV* human papillomavirus, *OR* odds ratio, *CI* confidence interval

P_c value = Bonferroni corrected *P* value. The correction number is 8

in comparison with controls, which remained significant after Bonferroni correction ($P_c = 0.004$ and 0.0007 for DPB1*13:01 and *05:01 alleles, respectively) (Table 1). However, the significance of increased DPB1*02:01 allele frequency (OR = 1.36, 95 % CI 1.02–1.81) in women with HPV-16 positive CSCC disappeared after Bonferroni correction ($P_c = 0.27$). For the +550 G/A polymorphism, no significant difference in genotype and allele distribution was observed between women with HPV-16 positive CSCC and controls (Table 2). LD analysis of the HLA-DPB1 variants between exon 2 alleles and +550 G/A SNP revealed high LDs in both controls and patients ($D' = 0.99$ and 0.95, respectively).

We also analyzed the possible haplotypes of HLA-DPB1 alleles and +550 G/A SNP in controls, women with CSCC, and those with HPV-16 positive CSCC (Table 3). Among the eight haplotypes, we found that haplotype frequencies of DPB1*02:01-A and DPB1*13:01-G increased significantly and DPB1*05:01-G decreased significantly in both CSCC patients (OR = 1.31, 95 % CI 1.03–1.67; OR = 1.55, 95 % CI 1.15–2.08; OR = 0.72, 95 % CI 0.61–0.85) and HPV-16 positive CSCC patients (OR = 1.51, 95 % CI 1.12–2.02; OR = 1.84, 95 % CI 1.30–2.62; OR = 0.63, 95 % CI 0.51–0.79), whereas DPB1*03:01-G frequency increased significantly (OR = 1.64, 95 % CI 1.07–2.53) only in HPV-16 positive CSCC patients as compared with controls. The significance remained for the DPB1*02:01-A haplotype ($P_c = 0.05$ for HPV-16 positive CSCC patients), DPB1*05:01-G haplotype ($P_c = 0.0009$ and 0.0004 for CSCC and HPV-16 positive CSCC patients, respectively) and DPB1*13:01-G haplotype ($P_c = 0.03$ and 0.004 for CSCC and HPV-16 positive CSCC patients, respectively) after Bonferroni correction.

Discussion

In the present study, we evaluated whether HLA-DPB1 variants and their haplotypes were associated with the susceptibility to cervical cancer in Taiwanese women. We found a decreased risk of CSCC in association with the HLA-DPB1*05:01 allele and +550 G/G genotype and G allele and an increased risk in association with the +550 A allele. When the analysis was restricted to the subgroup of women with HPV-16 positive CSCC, the association of HLA-DPB1*05:01 allele still existed and the DPB1*13:01 allele was shown to associate with CSCC risk. However, some nonsignificant associations such as DPB1*03:01 allele (OR = 1.52, 95 % CI 0.99–2.33), +550 A/A genotype (OR = 1.51, 95 % CI 0.97–2.36), and G (OR = 0.82, 95 % CI 0.65–1.02) and A (OR = 1.23, 95 % CI 0.98–1.54) alleles found in the HPV-16 subgroup could be due to the small sample size. In addition, DPB1*05:01-G and DPB1*13:01-G haplotypes were revealed to confer protection and risk in both CSCC patients and HPV-16 positive CSCC patients, respectively. Another risk haplotype, DPB1*02:01-A, was also found in the HPV-16 positive CSCC women. Our results suggest that the HLA-DPB1 gene plays a significant role in the pathogenesis of CSCC in Taiwanese women.

Certain HLA class II alleles have been reported to influence cervical cancer development by affecting the interactions between host immune response and HPV infection [23–26]. The DRB1 and DQB1 loci therefore have been thoroughly investigated in several cervical cancer association studies [5]. However, the HLA-DPB1 locus was seldom studied because of its few polymorphisms and low cell surface expression levels [27]. A significant increase in DPB1*13:01 allele in cervical cancer patients and a significant decrease in DPB1*04:01:01 allele in HPV-16 positive cervical cancer patients were previously reported in a central Chinese population [10]. Another study in southern Chinese women demonstrated an increased risk of cervical cancer with both HLA-DPB1*02:02 and *13:01 alleles [11]. In contrast, associations between HLA-DPB1 allele and cervical intraepithelial neoplasia were not found among southwestern American Indian women [9]. It is likely that differences in the study design, ethnicity, sample sizes, cancer types, and pertinent risk factors may result in the disconcordance between these studies and ours.

A number of genome-wide association studies have identified a SNP in the 3′ UTR of HLA-DPB1 gene (+550 G/A, dbSNP ID: rs 9277535), which was associated with chronic hepatitis B virus infection in various Asian populations [28–30]. These findings were validated subsequently in other studies [31–35]. The +550 G/A polymorphism has been reported to regulate HLA-DPB1 mRNA expression according to the mRNA levels obtained from both liver cells and macrophages [12]. Recently, Jiang et al. [36] also have investigated this SNP in cervical cancer and found that +550 GA, AA, and GA/AA genotypes were associated with cervical cancer susceptibility in Chinese women. Our findings of increased CSCC risk in association with +550 A allele in the current study are also consistent with the results of Jiang et al. [36].

The molecular mechanisms underlying the association between CSCC risk and HLA-DPB1 gene are still not well understood. However, on the basis of our results, it is reasonable to infer that HLA-DPB1*13:01 and +550 A alleles are able to affect the antigen presentation and cellular expression of HLA-DPB1 molecules. These specific HLA-DPB1 alleles therefore are likely to associate with persistent HPV infections and thus increase the risk of CSCC. If these associations can be replicated in different populations, it could help to provide better prevention and treatment strategies for women to carry these risk alleles. However, this study still has its limitations need to be considered. The correlations between HLA-DPB1 gene variants and viral load, length, and severity of the HPV infection cannot be addressed due to lack of these data. Furthermore, comparison between HPV-infected individuals with and without CSCC would better clarify the link between HPV infection, host immune response to the HPV and cervical cancer.

Our findings indicate that the HLA-DPB1*13:01 and +550 A alleles are significantly associated with CSCC risk in Taiwanese women. In addition, significant increase in CSCC risk was observed for the HLA-DPB1*13:01-G and DPB1*02:01-A haplotypes. Large-scale epidemiological studies are required to consolidate the HLA-DPB1 role in the development of cervical cancer.

Acknowledgments This research was supported by Grants MMH 9781 and MMH E-103-07 from Mackay Memorial Hospital, Taiwan.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D (2011) Global cancer statistics. CA Cancer J Clin 61:69–90
2. Chen CA, Hsieh CY (2004) Recent advances and problems in primary therapy for cervical cancer in Taiwan. J Formos Med Assoc 103:511–518
3. Munoz N (2000) Human papillomavirus and cancer: the epidemiological evidence. J Clin Virol 19:1–5
4. Magnusson PK, Sparen P, Gyllensten UB (1999) Genetic link to cervical tumours. Nature 400:29–30
5. Hildesheim A, Wang SS (2002) Host and viral genetics and risk of cervical cancer: a review. Virus Res 89:229–240

6. Madeleine MM, Johnson LG, Smith AG, Hansen JA, Nisperos BB, Li S, Zhao LP, Daling JR, Schwartz SM, Galloway DA (2008) Comprehensive analysis of HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQB1 loci and squamous cell cervical cancer risk. Cancer Res 68:3532–3539
7. Chan DP, Cheung TH, Tam AO, Cheung JL, Yim SF, Lo KW, Siu NS, Zhou DX, Chan PK (2005) Risk association between human leukocyte antigen-A allele and high-risk human papillomavirus infection for cervical neoplasia in Chinese women. J Infect Dis 192:1749–1756
8. Wang SS, Hildesheim A, Gao X, Schiffman M, Herrero R, Bratti MC, Sherman ME, Barnes WA, Greenberg MD, McGowan L, Mortel R, Schwartz PE, Zaino RJ, Glass AG, Burk RD, Karacki P, Carrington M (2002) Comprehensive analysis of human leukocyte antigen class I alleles and cervical neoplasia in 3 epidemiologic studies. J Infect Dis 186:598–605
9. Schiff MA, Apple RJ, Lin P, Nelson JL, Wheeler CM, Becker TM (2005) HLA alleles and risk of cervical intraepithelial neoplasia among southwestern American Indian women. Hum Immunol 66:1050–1056
10. Wu Y, Liu B, Lin W, Xu Y, Li L, Zhang Y, Chen S, Lin Z, Xu A (2007) Human leukocyte antigen class II alleles and risk of cervical cancer in China. Hum Immunol 68:192–200
11. Liang J, Xu A, Xie Y, Awonuga AO, Lin Z (2008) Some but not all of HLA-II alleles are associated with cervical cancer in Chinese women. Cancer Genet Cytogenet 187:95–100
12. O'Brien TR, Kohaar I, Pfeiffer RM, Maeder D, Yeager M, Schadt EE, Prokunina-Olsson L (2011) Risk alleles for chronic hepatitis B are associated with decreased mRNA expression of HLA-DPA1 and HLA-DPB1 in normal human liver. Genes Immun 12:428–433
13. Hildesheim A, Schiffman MH, Gravitt PE, Glass AG, Greer CE, Zhang T, Scott DR, Rush BB, Lawler P, Sherman ME et al (1994) Persistence of type-specific human papillomavirus infection among cytologically normal women. J Infect Dis 169:235–240
14. de Roda Husman AM, Walboomers JM, van den Brule AJ, Meijer CJ, Snijders PJ (1995) The use of general primers GP5 and GP6 elongated at their 3′ ends with adjacent highly conserved sequences improves human papillomavirus detection by PCR. J Gen Virol 76(Pt 4):1057–1062
15. van der Zwan AW, Rozemuller E, Tilanus MG (2006) Sequencing based typing for HLA-DPB1: Strategy for ABI equipment. In: Hansen JA (ed) Immunobiology of the human MHC. Proceedings of the 13th international histocompatibility workshop and conference. IHWG Press, Seattle, pp 374–375
16. Chu CC, Trejaut J, Lee HL, Lin M (2006) Anthropology/human genetic diversity population reports: Taiwan's populations. In: Hansen JA (ed) Immunobiology of the human MHC. Proceedings of the 13th international histocompatibility workshop and conference. IHWG Press, Seattle, pp 611–615
17. Meyer D, Single RM, Mack SJ, Lancaster A, Nelson MP, Erlich H, Fernandez-Vina M, Thomson G (2006) Single locus polymorphism of classical HLA genes. In: Hansen JA (ed) Immunobiology of the human MHC. Proceedings of the 13th international histocompatibility workshop and conference. IHWG Press, Seattle, pp 653–704
18. Lancaster AK, Single RM, Solberg OD, Nelson MP, Thomson G (2007) PyPop update—a software pipeline for large-scale multilocus population genomics. Tissue Antigens 69(Suppl 1):192–197
19. Guo SW, Thompson EA (1992) Performing the exact test of Hardy–Weinberg proportion for multiple alleles. Biometrics 48:361–372
20. Svejgaard A, Ryder LP (1994) HLA and disease associations: detecting the strongest association. Tissue Antigens 43:18–27
21. Ohashi J, Yamamoto S, Tsuchiya N, Hatta Y, Komata T, Matsushita M, Tokunaga K (2001) Comparison of statistical power between 2 * 2 allele frequency and allele positivity tables in case–control studies of complex disease genes. Ann Hum Genet 65:197–206
22. Chen YY, You SL, Chen CA, Shih LY, Koong SL, Chao KY, Hsiao ML, Hsieh CY, Chen CJ (2009) Effectiveness of national cervical cancer screening programme in Taiwan: 12-year experiences. Br J Cancer 101:174–177
23. Beskow AH, Josefsson AM, Gyllensten UB (2001) HLA class II alleles associated with infection by HPV16 in cervical cancer in situ. Int J Cancer 93:817–822
24. Beskow AH, Gyllensten UB (2002) Host genetic control of HPV 16 titer in carcinoma in situ of the cervix uteri. Int J Cancer 101:526–531
25. Beskow AH, Moberg M, Gyllensten UB (2005) HLA class II allele control of HPV load in carcinoma in situ of the cervix uteri. Int J Cancer 117:510–514
26. Chuang LC, Hu CY, Chen HC, Lin PJ, Lee B, Lin CY, Pan MH, You SL, Hsieh CY, Chen CJ (2012) Associations of human leukocyte antigen class II genotypes with human papillomavirus 18 infection and cervical intraepithelial neoplasia risk. Cancer 118:223–231
27. Edwards JA, Durant BM, Jones DB, Evans PR, Smith JL (1986) Differential expression of HLA class II antigens in fetal human spleen: relationship of HLA-DP, DQ, and DR to immunoglobulin expression. J Immunol 137:490–497
28. Kamatani Y, Wattanapokayakit S, Ochi H, Kawaguchi T, Takahashi A, Hosono N, Kubo M, Tsunoda T, Kamatani N, Kumada H, Puseenam A, Sura T, Daigo Y, Chayama K, Chantratita W, Nakamura Y, Matsuda K (2009) A genome-wide association study identifies variants in the HLA-DP locus associated with chronic hepatitis B in Asians. Nat Genet 41:591–595
29. Mbarek H, Ochi H, Urabe Y, Kumar V, Kubo M, Hosono N, Takahashi A, Kamatani Y, Miki D, Abe H, Tsunoda T, Kamatani N, Chayama K, Nakamura Y, Matsuda K (2011) A genome-wide association study of chronic hepatitis B identified novel risk locus in a Japanese population. Hum Mol Genet 20:3884–3892
30. Nishida N, Sawai H, Matsuura K, Sugiyama M, Ahn SH, Park JY, Hige S, Kang JH, Suzuki K, Kurosaki M, Asahina Y, Mochida S, Watanabe M, Tanaka E, Honda M, Kaneko S, Orito E, Itoh Y, Mita E, Tamori A, Murawaki Y, Hiasa Y, Sakaida I, Korenaga M, Hino K, Ide T, Kawashima M, Mawatari Y, Sageshima M, Ogasawara Y, Koike A, Izumi N, Han KH, Tanaka Y, Tokunaga K, Mizokami M (2012) Genome-wide association study confirming association of HLA-DP with protection against chronic hepatitis B and viral clearance in Japanese and Korean. PLoS One 7:e39175
31. Guo X, Zhang Y, Li J, Ma J, Wei Z, Tan W, O'Brien SJ (2011) Strong influence of human leukocyte antigen (HLA)-DP gene variants on development of persistent chronic hepatitis B virus carriers in the Han Chinese population. Hepatology 53:422–428
32. Wang L, Wu XP, Zhang W, Zhu DH, Wang Y, Li YP, Tian Y, Li RC, Li Z, Zhu X, Li JH, Cai J, Liu L, Miao XP, Liu Y, Li H (2011) Evaluation of genetic susceptibility loci for chronic hepatitis B in Chinese: two independent case–control studies. PLoS ONE 6:e17608
33. Li J, Yang D, He Y, Wang M, Wen Z, Liu L, Yao J, Matsuda K, Nakamura Y, Yu J, Jiang X, Sun S, Liu Q, Song Q, Chen M, Yang H, Tang F, Hu X, Wang J, Chang Y, He X, Chen Y, Lin J (2011) Associations of HLA-DP variants with hepatitis B virus

infection in southern and northern Han Chinese populations: a multicenter case–control study. PLoS One 6:e24221
34. Hu L, Zhai X, Liu J, Chu M, Pan S, Jiang J, Zhang Y, Wang H, Chen J, Shen H, Hu Z (2012) Genetic variants in human leukocyte antigen/DP-DQ influence both hepatitis B virus clearance and hepatocellular carcinoma development. Hepatology 55:1426–1431
35. Al-Qahtani AA, Al-Anazi MR, Abdo AA, Sanai FM, Al-Hamoudi W, Alswat KA, Al-Ashgar HI, Khalaf NZ, Eldali AM, Viswan NA, Al-Ahdal MN (2014) Association between HLA variations and chronic hepatitis B virus infection in Saudi Arabian patients. PLoS One 9:e80445
36. Jiang J, Li N, Shen Y, Liu J, Liu L, Du J, Lei Y, Wen Y, Yang L, Guo L, Zhang K, Qiang F, Wang S, Hu Z, Dai M, Shen H (2013) Genetic variants in HLA-DP/DQ contribute to risk of cervical cancer: a two-stage study in Chinese women. Gynecol Oncol 129:401–405