Selected class I and class II HLA alleles and haplotypes and risk of high-grade cervical intraepithelial neoplasia

Steven Ades[1,2], Anita Koushik[1,3], Eliane Duarte-Franco[2], Nabil Mansour[4,5], Jocelyne Arseneau[5], Diane Provencher[6], Lucy Gilbert[7], Walter Gotlieb[4,5], Alex Ferenczy[4,5], François Coutlée[2,8], Michel Roger[8] and Eduardo L. Franco[1,2]* for the Biomarkers of Cervical Cancer Risk (BCCR) Study Team

[1]*Department of Epidemiology and Biostatistics, McGill University, Montréal, Canada*
[2]*Department of Oncology, McGill University, Montréal, Canada*
[3]*Department of Social and Preventive Medicine, Université de Montréal, Montréal, Canada*
[4]*Department of Obstetrics and Gynecology, Sir Mortimer B. Davis-Jewish General Hospital, Montréal, Canada*
[5]*Department of Pathology, Sir Mortimer B. Davis-Jewish General Hospital, Montréal, Canada*
[6]*Department of Gynaecology, Centre Hospitalier de l'Université de Montréal (CHUM), Université de Montréal, Montréal, Canada*
[7]*Department of Gynaecology and Obstetrics, McGill University, Montréal, Canada*
[8]*Department of Microbiology and Infectious Diseases, Centre Hospitalier de l'Université de Montréal (CHUM), Université de Montréal, Montréal, Canada*

Human leukocyte antigens (HLAs) present foreign antigens to the immune system and may be important determinants of cervical neoplasia. Previously published associations between HLA and cervical neoplasia exhibit considerable variation in findings. The biomarkers of cervical cancer risk (BCCR) case-control study addressed the role of specific HLA alleles as cofactors in the development of high-grade cervical intraepithelial neoplasia (HG-CIN) based on the most consistent evidence from published literature. Cases ($N = 381$) were women with histologically-confirmed HG-CIN attending colposcopy clinics and controls ($N = 884$) were women from outpatient clinics with normal cytological screening smears. Subjects were mainly of French-Canadian descent. Cervical specimens were tested for human papillomavirus (HPV) DNA and HLA genotypes by PGMY L1 consensus primer PCR and a PCR sequence-specific primer method, respectively. Unlike other studies, the DQB1*03 and DRB1*13 allele groups were not associated with risk of HG-CIN. The B7-DRB1*1501-DQB1*0602 haplotype was associated with a 41% overall reduction in HG-CIN risk (odds ratio [OR] = 0.59; 95% confidence interval [CI]: 0.36–0.96), and an 83% reduction in risk of HG-CIN among HPV 16 or HPV 18-positive subjects (OR = 0.17; 95%CI: 0.05–0.54). Paradoxically, however, the same haplotype was associated with HPV 16/18 infection risk among controls (OR = 8.44, 95%CI: 1.12–63.73). In conclusion, the B7-DRB1*1501-DQB1*0602 haplotype was protective against HG-CIN, especially in individuals infected with oncogenic HPV, but the mechanism of the association seems to involve multiple steps in the natural history of HPV and CIN.

Key words: cervical intraepithelial neoplasia; human leukocyte antigen; human papillomavirus; case-control study

After breast cancer, cervical cancer is the second most common cancer in women worldwide, with 493,000 new cases and over 273,000 deaths annually.[1] It is well established that the cervical infection caused by one of up to 18 oncogenic human papillomaviruses (HPV) is the main causal factor in cervical cancer, with HPV 16 alone accounting for over half of all cases.[2] In fact, HPV infection can be considered a necessary intermediate step in cervical carcinogenesis.[3] On the other hand, despite the presence of asymptomatic cervical HPV infection in 5–50% of women of reproductive age,[4,5] only a small fraction of infected women will develop cervical cancer. Thus, HPV infection alone is not a sufficient cause of cervical cancer, which underscores the need to identify cofactors that may mediate risk of persistent HPV infection or progression to cervical dysplastic changes and overt neoplasia. The human leukocyte antigens (HLA) mediate the presentation of HPV antigens to the immune system and thus may either predispose to or protect against development of HPV-associated cervical neoplasia.[6]

Previous case-control studies examining the relationship have suggested that certain HLA polymorphisms are associated with risk of neoplastic transformation, but have yielded contradictory results possibly because of the variations in study design, ethnic diversity of target populations, lack of adjustment for known risk factors, modest sample sizes, and issues related to multiple significance testing in the search for associations between large numbers of alleles and risk of neoplasia. The most consistent published evidence to date involves the HLA-DQB1*03 (DQ3) allele and HLA-DRB1*1501-DQB1*0602 haplotype. Increased risks (approximately 2-fold) for cervical cancer or malignant precursor lesions have been identified in different populations for HLA-DQB1*03 (DQ3) allele[7–13] or HLA-DRB1*1501-DQB1*0602[12,14–17] haplotype carriers. Other studies did not show any association with HLA-DQB1*03[15–19] or HLA-DRB1*1501-DQB1*0602.[10,11,13,18–20] One large study conversely reporting a significant protective association for the HLA-DRB1*1501-DQB1*0602 haplotype.[21] More consistent findings have been reported in favor of a protective effect (odds ratios (ORs) of approximately 0.3–0.4) for HLA alleles DRB1*13 and DQB1*0603, which are in linkage disequilibrium.[10–15,19,20] Two larger studies in distinct populations in the US and Costa Rica showed elevated risks of low- and high-grade cervical lesions and cancer in women who were carriers of both class I allele HLA-B7 and DQB1*0302.[19,21]

The Biomarkers of Cervical Cancer Risk (BCCR) case-control study was designed to investigate the role of the several candidate

alleles from Class I and Class II genes on the risk of preinvasive high grade cervical neoplasia in a Canadian population, after adjusting for the potential mediating or confounding effects of ethnicity and other known risk factors.

Material and methods

Study design and recruitment

The BCCR study employed a case-control design. Cases consisted of patients referred with abnormal screening Pap smears to one of 5 colposcopy clinics in Montreal belonging to one of 2 university hospital centers. Participants were recruited between February 2001 and January 2004. Women were invited to participate as cases if at the time of colposcopy they required a cervical biopsy. The case group was defined as women who had high-grade CIN classified as moderate to severe dysplasia (CIN 2 or 3) or early invasive cancers (T1a or T1b). A cervical specimen was obtained for HLA and HPV testing prior to biopsy. Histological diagnosis meeting criteria for case definition was necessary for inclusion in the study. Controls were recruited during the same time period from among women presenting for their annual routine Pap smear test at family medicine and gynecology centers that referred women to the collaborating hospitals, with frequency matching by age (± 5 years) and hospital. The control group was defined as women with a normal screening Pap smear and without any history of cervical neoplasia or diagnosis of cervical abnormalities. In the interest of maximizing precision, we attempted to recruit twice as many controls as cases were identified. Required cervical specimens for the study were obtained in addition to the Pap smear. The participant was only included in the final analysis if the screening smear was normal or indicated only benign cellular changes.

For both potential cases and controls, informed consent was obtained and relevant personal information was obtained through a self-administered questionnaire. The questionnaire requested information on ethnic background, sociodemographics, smoking habits, reproductive history, use of contraceptives and sexual practices. This questionnaire is a subset of another that was used in 2 separate studies on risk factors for HPV, conducted in the Montreal area.[22,23] Exclusion criteria for both cases and controls included current pregnancy, prior hysterectomy or conization surgery, prior history of any cervical abnormalities and prior history of any cancer, except nonmelanoma skin cancer.

The BCCR study protocol and informed consent forms for both cases and controls were approved by the local ethics review boards of all 5 participating hospitals, the McGill Institutional Review Board, and the Comité d'éthique de la recherche du Centre Hospitalier de l'Université de Montréal (CHUM) prior to commencement of recruitment. All subjects provided written informed consent.

Cervical specimen processing

Specimens containing ectocervical and endocervical cells were collected from both cases and controls. The study nurse collected exfoliated cervical cells using an Accelon biosampler (Medscand, Inc., Hollywood, FL) that were resuspended in Preservcyt (Cytyc Corporation, Boxborough, MA) for DNA extraction. A Pap smear was prepared onto a glass slide and fixed in 95% ethanol. One milliliter of the cell suspension in Preservcyt was centrifuged at 13,000*g* for 15 min at 22°C, the supernatant was discarded, and the cell pellet was dried. Pellets were resuspended into 100 µl of 10 mM Tris-EDTA buffer pH 8.3, and DNA was purified using the Master pure procedure.[24] A 268-base pair segment of the human β-globin gene was amplified to confirm the presence of DNA and specimen integrity.[25] Each cervical DNA specimen was then divided into 2 aliquots. One was retained for HPV analyses and the second sent to HLA typing.

HLA typing

Specific HLA alleles were genotyped using a PCR sequence specific primers (PCR-SSP) method. Samples were tested for the presence of class I B7 and class II DQB1*03, DRB1*13 and DRB1*15 alleles in multiplex PCR reactions using the primer pairs and conditions as previously described.[26] High-resolution typing for DQB1*0602 and DRB1*1501 alleles was done in 2 separate PCR reactions using the sequence specific primers and conditions as previously described.[21,27] Amplification control primers giving rise to a 796-base pair fragment from the third intron of HLA-DRB1 were included in PCR reactions to verify successful amplification.[26] Equivocal test results were repeated for confirmation.

HPV testing

β-globin-positive specimens were tested for the presence of HPV DNA with PCR employing the widely used PGMY09/11 primer set.[28] Negative, weak positive and strong positive controls were included in each amplification experiment. HPV typing of the amplified products was performed using the reverse line blot assay.[29] Thirty-six genital HPV genotypes were detected with this technique: 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, 89 and the IS39 subtype of HPV 82. These HPV types were grouped according to putative oncogenicity as per a recent epidemiologic classification.[2]

Statistical analysis

All descriptive and regression model statistics were performed using Stata version 8.2 (StataCorp, College Station, TX). The denominator for this analysis consists of all participants who have complete information on a specific candidate allele. Appropriate descriptive statistics (means and proportions) were generated for all relevant variables. To assess potential linkage disequilibrium between 2 alleles, we calculated correlation coefficients (r). The association between each of the putative HLA alleles (or haplotypes) and risk of high-grade preinvasive cervical cancer (HG-CIN) was investigated using crude and adjusted multivariate logistic regression to derive OR and respective 95% confidence intervals (CI) as estimates of relative risks. Specifically, the models were adjusted in incremental steps for age, ethnicity and HPV infection status as a four-level, hierarchically-defined ordinal variable (negative, only low-risk, any high-risk type other than HPV 16 or HPV 18, and HPV 16 or 18 positivity, regardless of other types).

Women's race was initially classified according to continental categories,[30] based on questionnaire information regarding ethnic group/country of origin of both parents. In the final analysis, participants of African, Asian, Pacific Islander and Native American ancestry were combined with the other/mixed race category due to the paucity of numbers. This left 3 ethnic categories for statistical analysis: French Canadian (reference), other Caucasians, and other/admixed races.

An exploratory analysis using 3 different subsets of HPV-infected controls was performed to provide insight into the role of these alleles and haplotypes in altering progression to carcinoma in infected women. The model was adjusted using a forward-selection strategy and a change-in-estimate criterion of 10% for inclusion of covariates to be controlled for in the analysis. A second subset analysis restricted to controls only was performed to provide insights into the possible role of these alleles and haplotypes in HPV infection acquisition and persistence. HPV positivity served as the dependent variable in the latter analysis.

Results

Description of study population

Between February 2001 and January 2004, 605 potential cases and 1,129 controls, were approached in participating clinics. Of these, 590 and 1,097 women agreed to participate, respectively. Among these participants, 396 (67.1%) potential case and 932 (85.0%) potential control subjects were identified as eligible. One hundred and ninety-four potential cases were excluded for one of 2 main reasons: (*i*) biopsy did not meet case criteria (54 LG-CIN, 32 normal, 19 chronic cervicitis, 7 equivocal, 7 squamous metaplasia,

TABLE I – DISTRIBUTION OF CASES AND CONTROLS ACCORDING TO SOCIODEMOGRAPHIC, REPRODUCTIVE AND SEXUAL BEHAVIOR CHARACTERISTICS[1]

Variable	Category	Cases (N = 381)		Controls (N = 884)	
		N	%	*N*	%
Age (years)	<20	11	2.9	56	6.3
	20–24	77	20.2	205	23.2
	25–29	80	21.0	197	22.3
	30–34	72	18.9	165	18.7
	35–39	72	18.9	102	11.5
	>39	69	18.1	158	17.9
	Mean (SD)	32.0 (9.1)		31.2 (10.0)	
Race	French Canadian	219	57.5	502	56.8
	Caucasian[2]	91	23.9	198	22.4
	African	15	3.9	25	2.8
	Asian	8	2.1	20	2.3
	Other	45	11.8	134	15.2
Smoking	Never	128	33.6	410	46.4
	Former	90	23.6	196	22.2
	Current	154	40.4	262	29.6
Alcohol consumption	≥1 drink per week	252	66.1	509	57.6
	<1 drink per week	124	32.5	367	41.5
Socio-economic status	Difficult	40	10.5	63	7.1
	Moderate	193	50.7	405	45.8
	Comfortable	142	37.3	398	45.0
Highest level of education	Elementary	5	1.3	10	1.1
	Secondary	102	26.8	192	21.7
	Post-Secondary	270	70.9	678	76.7
Marital status	Married/stable partner	166	43.6	432	48.9
	Other	211	55.4	447	50.6
Age at 1st intercourse	Mean (SD)	17.0 (3.0)		17.4 (3.2)	
Lifetime number of male vaginal intercourse partners	<3	58	15.2	246	27.8
	3–5	97	25.5	246	27.8
	6–9	74	19.4	152	17.2
	10–20	113	29.7	189	21.4
	>20	32	8.4	35	4.0
	Mean (SD)	10.0 (11.4)		7.2 (9.8)	
	Mean in last year (SD)	1.5 (1.4)		1.4 (2.0)	
Oral contraceptive use	Never	36	9.4	83	9.4
	Regular	209	54.9	513	58.0
	Currently	161	42.3	387	43.8
	Mean age at start (SD)	18.1 (4.2)		18.2 (3.8)	
Previous pregnancy	Never	147	38.6	420	47.5
	Ever	230	60.4	457	51.7
	Mean (SD)	1.5	(2.0)	1.2	(1.5)
HPV negative		15	3.9	551	62.3
Low-risk HPV	Positive	102	26.8	168	19.0
	Negative	273	71.7	647	73.2
High-risk HPV[3]	Positive	354	92.9	174	19.7
	Negative	21	5.5	641	72.5

[1]Numbers may not add up to totals in each group due to missing data.–[2]Other than French Canadians.–[3]According to Munoz *et al*.[2] Includes HPV16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73 and 82.

5 adenocarcinoma, 3 condylomata, 1 parakeratosis and 8 pending at the time of analysis); and (*ii*) did not meet other eligibility criteria (58 women) because of failure to complete the questionnaire, biopsy not performed or submitted, or pregnancy. One hundred and sixty-five potential controls were excluded for 1 of 2 main reasons: (*i*) cytology did not meet control criteria (59 ASCUS, 53 pending at the time of the analysis, 6 lost specimens and 3 unsatisfactory specimens); and (*ii*) did not meet other criteria (44 women), most commonly a prior history of cervical abnormalities. For the analyses, 381 (96.1%) and 884 (94.8%) of confirmed case and control subjects had completed HLA analyses, respectively. No cases and only 3 subjects in the control group had inadequate specimens for HLA typing. The case group comprised of women with HG-CIN except for 2 cases of microinvasive carcinoma.

Table I shows the distribution of cases and controls according to sociodemographic, reproductive and behavioral characteristics, cigarette and alcohol consumption and HPV carrier status. The age and racial distributions were similar between cases and controls with most participants being either French Canadian or other Caucasian. For all other variables shown, differences between cases and controls were mostly reflective of the expected direction of the association with known cervical cancer risk factors (*i.e*., increased lifetime number of intercourse partners, smoking, alcohol consumption, lower socioeconomic status, lower education level, lower marital rate and higher pregnancy rate).

Description of HLA polymorphisms

Table II shows the proportions of cases and controls possessing specific alleles and haplotypes. The DRB1*1501 and DQB1*0602 alleles were in linkage disequilibrium (r = 0.95). HLA-B7 was mildly correlated with both DRB1*1501 (r = 0.34) and DQB1*0602 (r = 0.33). The proportions of subjects harboring any of the alleles or haplotypes were largely comparable between cases and controls, with the exception of B7-DRB1*1501 (6.8 *vs*. 10.2%; p = 0.05) and B7-DQB1*0602 (5.8 *vs*. 9.7%; p = 0.02). The allele combination of B7 and DQB1*0302 was not in linkage disequilibrium in the BCCR study population (r = −0.044). There were comparable proportions of cases and controls who possessed this allele combination (9.7 *vs*. 9.0%).

Table III shows the analyses of the associations between HLA alleles and haplotypes and HG-CIN. All analyses were based on a crude logistic regression model and 2 levels of incremental adjust-

ment: (*i*) for age and ethnicity alone and (*ii*) for age, ethnicity and HPV infection status. No differences were noted in the crude model for most polymorphisms, but protective associations were found for B7-containing haplotypes. Adjustment for age and ethnicity produced only a small change in ORs of HG-CIN for most polymorphisms. However, on further adjustment for HPV infection status, the borderline protective associations for HLA-B7, DRB1*1501, DQB1*0602, DRB1*1501-DQB1*0602 and the 2 B7-containing haplotypes became more pronounced. B7-containing haplotypes were associated with more than halving of the risk of HG-CIN after adjustment for HPV status. Results for the same models, but restricted to French Canadian and Caucasian women, and to French Canadian women alone were similar to those for all cases and controls (data not shown).

HPV-restricted subsets

Table IV shows the results of the analyses for subsets restricted to HPV-positive subjects with a view to examining a potential role for the HLA polymorphisms "downstream" from HPV infection. Three incrementally selective definitions of HPV positivity were chosen for the analysis. Aside from the crude analysis, regression models were incrementally adjusted in 2 stages: (*i*) for age and ethnicity; (*ii*) for age, ethnicity and empirically selected covariates for progression to HG-CIN rather than HPV infection acquisition in these HPV-positive subsets (see statistical analysis). Women with HLA-B7, DRB*1501 or DQB1*0602 had moderately lower risks of HG-CIN for analyses restricted to oncogenic HPV positive subjects and HPV 16/18-positive subjects. Although there appears to be a trend for protective alleles to become less so after adjustments, none of the associations are statistically significant except for crude haplotype associations with HG-CIN risk.

In analogy with the global model involving all HG-CIN cases and all controls, significantly reduced risks of disease were observed for all B7-containing haplotypes, particularly after adjustment for age, ethnicity and empirical confounders. The OR of having HG-CIN for the B7-DRB1*1501-DQB1*0602 haplotype in the HPV 16/18-positive subset was 0.17 (95%CI: 0.05–0.54). The proportion of HPV 16/18-positive women possessing the B7-DRB1*1501-DQB1*0602 haplotype in the case group was only 6.4% (14/219), compared to 23.4% (11/47) of controls. After further adjustment for the presence of B7 and DRB1*1501-DQB1*0602, among both high-oncogenic risk HPV subsets the composite B7-DRB1*1501-DQB1*0602 haplotype remained significant and very protective, but only 14 of the 219 cases who were HPV 16 or 18 positive also possessed the B7-DRB1*1501-DQB1*0602 haplotype. Of note, the distribution of racial groups for all 111 women who possessed the protective B7-DRB1*1501-DQB1*0602 haplotype was similar to the overall distribution (64.0% *vs.* 57.1% French Canadian women) and results for the same models, but restricted to French Canadian and Caucasian women were similar to those for all cases and controls (data not shown).

TABLE II – DISTRIBUTION OF CASES AND CONTROLS ACCORDING TO HLA ALLELES AND HAPLOTYPES

Variable	Cases (381)		Controls (884)	
	N	%	*N*	%
B7	60	15.7	164	18.6
DQB1*03	225	59.1	497	56.2
DRB1*13	92	24.1	227	25.7
DRB1*1501	73	19.2	197	22.3
DQB1*0602	68	17.8	182	20.6
DRB1*1501-DQB1*0602	68	17.8	181	20.5
B7-DRB1*1501[1]	26	6.8	90	10.2
B7-DQB1*0602[2]	22	5.8	86	9.7
B7-DRB1*1501-DQB1*0602	22	5.8	85	9.6

[1] $p = 0.0546$.–[2] $p = 0.0227$.

HLA-HPV associations among control subjects

In the following analyses, cases were excluded and HPV status was used as outcome variable among controls exclusively. HPV outcome status was based on 3 different definitions of HPV positivity: any HPV type, oncogenic HPV and HPV 16 or 18. These different definitions of HPV positivity served to create binary dependent variables for logistic regression models.

The B7, DRB1*1501 and DQB1*0602 alleles were associated with increased risk of being infected with HPV 16 or HPV 18, while DRB*13 seemed modestly protective (Table V). Significant tripling of the risks of HPV 16/18 positivity was observed for all B7-containing haplotypes. The OR for the composite B7-DRB1*1501-DQB1*0602 haplotype was 2.87 (95%CI: 1.38–5.97). When the association between B7 and risk of HPV 16/18 infection was adjusted for the presence of the DRB1*1501-DQB1*0602 and B7-DRB1*1501-DQB1*0602 haplotypes, it was magnified and significant for B7-DRB1*1501-DQB1*0602 (OR = 8.44, 95%CI: 1.12–63.73), while the increased risks of infection for both B7 and DRB1*1501-DQB1*0602 disappeared. The low precision in measuring the latter association is due to the small absolute number of women infected with HPV16 or HPV18 and possessing the B7-DRB1*1501-DQB1*0602 haplotype in the control group.

Discussion

In contrast to most other studies, which genotyped for large arrays of alleles, the limited number of comparisons in the BCCR study lowered the likelihood of detecting falsely positive associations. An important limitation is the misclassification of cumulative HPV exposure inherent to the case-control study design. This impacts on the ability to address the role of potential cofactors such as HLA genotype at specific steps in cervical carcinogenesis.[31]

A potential concern for studies of genetic factors and cancer risk is the impact of population stratification as a source of con-

TABLE III – ASSOCIATIONS BETWEEN INDIVIDUAL HLA ALLELES AND HAPLOTYPES AND HG-CIN RISK (381 CASES, 884 CONTROLS)

HLA allele or haplotype	Odds ratio (95% confidence interval)		
	Crude	Adjusted[1]	Adjusted[2]
B7	0.81 (0.59–1.13)	0.81 (0.58–1.12)	0.57 (0.35–0.92)
DQB1*03	1.11 (0.87–1.42)	1.12 (0.88–1.44)	1.26 (0.86–1.83)
DRB1*13	0.92 (0.69–1.21)	0.92 (0.70–1.22)	1.40 (0.92–2.15)
DRB1*1501	0.82 (0.61–1.11)	0.83 (0.61–1.13)	0.66 (0.42–1.04)
DQB1*0602	0.83 (0.61–1.14)	0.86 (0.63–1.17)	0.64 (0.40–1.03)
DRB1*1501-DQB1*0602	0.84 (0.62–1.14)	0.86 (0.63–1.18)	0.67 (0.42–1.07)
B7-DRB1*1501	0.65 (0.41–1.02)	0.63 (0.40–1.00)	0.42 (0.22–0.79)
B7-DQB1*0602	0.57 (0.35–0.92)	0.58 (0.36–0.95)	0.36 (0.19–0.71)
B7-DRB1*1501-DQB1*0602	0.57 (0.35–0.93)	0.59 (0.36–0.96)	0.39 (0.20–0.76)

[1]Adjusted for age (tertiles) and ethnicity (indicator variable set including French Canadians, other Caucasians, and all other ethnic backgrounds (see text).–[2]Adjusted for above variables and incrementally for HPV infection status (3 regressors indicating an ordinal score with the following 4 levels: HPV negative, low-risk HPV types, high-risk HPV and HPV16 or HPV18).

TABLE IV – ASSOCIATIONS BETWEEN INDIVIDUAL HLA ALLELES AND HAPLOTYPES AND HG-CIN RISK IN DIFFERENT SUBSETS RESTRICTED TO HPV POSITIVE SUBJECTS[1]

Subset and allele	Odds ratio (95% confidence interval)		
	Crude	Adjusted[2]	Adjusted[3]
Positive for any HPVs (381 cases, 264 controls)			
B7	0.74 (0.49–1.12)	0.77 (0.51–1.18)	0.90 (0.53–1.53)
DQB1*03	1.20 (0.88–1.65)	1.27 (0.92–1.77)	1.17 (0.77–1.77)
DRB1*13	0.97 (0.68–1.40)	1.02 (0.70–1.49)	1.18 (0.73–1.92)
DRB1*1501	0.82 (0.56–1.21)	0.86 (0.58–1.28)	1.00 (0.61–1.65)
DQB1*0602	0.81 (0.54–1.20)	0.86 (0.57–1.29)	1.00 (0.60–1.67)
DRB1*1501-DQB1*0602	0.83 (0.56–1.23)	0.88 (0.58–1.32)	1.00 (0.60–1.67)
B7-DRB1*1501	0.57 (0.33–0.99)	0.59 (0.34–1.05)	0.76 (0.38–1.52)
B7-DQB1*0602	0.50 (0.28–0.89)	0.56 (0.31–1.00)	0.70 (0.35–1.42)
B7-DRB1*1501-DQB1*0602	0.52 (0.29–0.92)	0.58 (0.32–1.04)	0.70 (0.35–1.42)
Positive for oncogenic HPVs[4] (381 cases, 174 controls)			
B7	0.63 (0.40–0.98)	0.67 (0.42–1.07)	0.73 (0.41–1.31)
DQB1*03	1.17 (0.82–1.68)	1.29 (0.88–1.88)	1.17 (0.72–1.88)
DRB1*13	0.94 (0.62–1.42)	1.04 (0.68–1.61)	1.20 (0.69–2.07)
DRB1*1501	0.68 (0.44–1.04)	0.70 (0.45–1.09)	0.90 (0.51–1.56)
DQB1*0602	0.66 (0.43–1.02)	0.69 (0.44–1.09)	0.92 (0.52–1.62)
DRB1*1501-DQB1*0602	0.68 (0.44–1.06)	0.71 (0.45–1.12)	0.92 (0.52–1.62)
B7-DRB1*1501	0.44 (0.24–0.78)	0.48 (0.26–0.87)	0.64 (0.31–1.34)
B7-DQB1*0602	0.37 (0.20–0.67)	0.43 (0.23–0.80)	0.59 (0.28–1.25)
B7-DRB1*1501-DQB1*0602	0.38 (0.21–0.70)	0.45 (0.24–0.84)	0.59 (0.28–1.25)
Positive for HPV16 or HPV18 (219 cases, 47 controls)			
B7	0.59 (0.28–1.22)	0.62 (0.29–1.31)	0.53 (0.21–1.36)
DQB1*03	1.53 (0.81–2.89)	1.54 (0.80–2.96)	1.37 (0.59–3.18)
DRB1*13	1.16 (0.50–2.66)	1.19 (0.51–2.79)	2.90 (0.76–11.12)
DRB1*1501	0.64 (0.32–1.30)	0.63 (0.31–1.31)	0.58 (0.24–1.45)
DQB1*0602	0.54 (0.27–1.08)	0.53 (0.26–1.09)	0.52 (0.21–1.31)
DRB1*1501-DQB1*0602	0.59 (0.29–1.20)	0.58 (0.28–1.21)	0.52 (0.21–1.31)
B7-DRB1*1501	0.26 (0.11–0.60)	0.27 (0.11–0.65)	0.20 (0.07–0.61)
B7-DQB1*0602	0.20 (0.09–0.47)	0.21 (0.09–0.52)	0.17 (0.05–0.54)
B7-DRB1*1501-DQB1*0602	0.22 (0.09–0.53)	0.24 (0.10–0.59)	0.17 (0.05–0.54)

[1]For the first 2 subsets, all cases and only controls who were HPV-positive or high-risk HPV positive, respectively, were included. In the third subset, only cases and controls who were HPV16 or HPV18 positive were selected.–[2]Adjusted for age (tertiles) and ethnicity (indicator variable set including French Canadians, other Caucasians, and all other ethnic backgrounds).–[3]Adjusted for variables in the 2nd footnote, plus empirical adjustment for hospital center, number of live births, number of sexual partners in the previous year, number of recent sexual partners that were new, alcohol consumption and previous history of any sexually transmitted disease.–[4]According to Munoz *et al*.[2] Includes HPV16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82 (including IS39).

founding[32,33] and type I error.[34] We attempted to control for these biases including frequency matching by hospital and adjustment for race, with inclusion of French Canadian participants as a separate ethnicity category. French Canadian women may differ from other Caucasian participants in terms of unmeasured genetic, environmental, or lifestyle-related risk factors, and the potential for bias exists when ethnicity is not adjusted for in the analysis.[35] The distributions of racial categories between cases and controls appeared similar making population stratification unlikely. It is also reassuring that there were also minimal nonsignificant differences between most crude and adjusted ORs, and restricting the analyses to the predominant French-Canadian subset yielded similar results.

In the control group, 32.4% of participants were infected with any HPV type and 18.7% were infected with high-oncogenic risk HPV types. The overall and age-specific prevalences were higher than corresponding figures from an Ontario cohort[36] (13.3 and 9.6%, respectively). This difference partly reflects a higher number of lifetime intercourse partners and the use of the more sensitive PGMY09/11 PCR system for HPV detection in our study.

Allele combinations are designated as haplotypes when there is evidence of linkage disequilibrium, and serve as markers of comparability across populations.[37] The HLA allele distributions were mostly comparable to those of other predominantly Caucasian populations.[10,21] To our knowledge, the distribution of these alleles in the French-Canadian population has not been reported. A case-control study in Portland, Oregon also found DRB1*1501-DQB1*0602 to be highly correlated ($r = 0.90$) and HLA-B7 to be correlated with DRB1*1501-DQB1*0602 ($r = 0.45$).[10] In contrast, the DRB1*1501 and DQB1*0602 were only moderately correlated ($r = 0.60$) and there was no correlation between HLA-B7 and DRB1*1501-DQB1*0602 in a study from Costa Rica.[19]

In contrast to some previous studies[12,15,17,38] but in agreement with others,[10,11,13,19] there was no evidence of increased risk due to DRB1*1501-DQB1*0602. Only a single study examining the role of HLA and CIN has shown a significant protective effect for DRB1*1501-DQB1*0602.[21] In the HPV-adjusted analysis and in the analysis restricted to HPV 16/18-positive cases and controls, this haplotype was mildly protective. When adjusted for the presence of the composite B7-DRB1*1501-DQB1*0602 haplotype, the protective effect disappeared, suggesting that the key association is confined to women possessing B7-DRB1*1501-DQB1*0602. The heterogeneity in results for this haplotype across studies and in different ethnic populations suggests that DRB1*1501-DQB1*0602 is not a host cofactor, which directly modifies the risk of HG-CIN. The role of DRB1*1501-DQB1*0602 is more likely race-specific, resulting from linkage disequilibrium with other causal alleles in certain populations such as American Hispanics[14,38] and Swedes.[12,15]

The Class I HLA-B7 allele was not associated in the BCCR study either with a weak risk of HG-CIN (for the allele alone), or with a higher risk of disease when coinherited together with DQB1*0302 as has been reported recently in 2 other studies.[19,21] Similar to DRB1*1501-DQB1*0602, a nonsignificant weakly protective effect for the allele in the HPV-adjusted global analysis and in the HPV-restricted analyses disappeared after adjustment for the presence of the B7-DRB1*1501-DQB1*0602 haplotype. A recent comprehensive analysis of Class I alleles and cervical neoplasia using high-resolution HLA genotypic assays did not report a significant risk-conferring effect for HLA-B*0702.[39] Reduction

TABLE V – ODDS RATIOS OF HPV POSITIVITY ASSOCIATED WITH SPECIFIC ALLELES AND HAPLOTYPES AMONG CONTROLS

Outcome and allele	Odds ratio (95% confidence interval)	
	Crude	Adjusted[1]
Positive for any HPV type (264 positive controls, 551 negative controls)		
B7	1.13 (0.78–1.64)	1.07 (0.73–1.57)
DQB1*03	0.94 (0.70–1.26)	0.90 (0.66–1.22)
DRB1*13	0.94 (0.67–1.32)	0.92 (0.64–1.30)
DRB1*1501	1.01 (0.71–1.44)	0.92 (0.64–1.33)
DQB1*0602	1.08 (0.75–1.55)	0.96 (0.66–1.39)
DRB1*1501-DQB1*0602	1.06 (0.73–1.52)	0.94 (0.64–1.36)
B7-DRB1*1501	1.13 (0.71–1.81)	1.04 (0.64–1.69)
B7-DQB1*0602	1.16 (0.72–1.87)	1.05 (0.64–1.73)
B7-DRB1*1501-DQB1*0602	1.11 (0.69–1.81)	1.01 (0.62–1.67)
Positive for oncogenic HPV[2] (174 positive controls, 641 negative controls)		
B7	1.21 (0.75–1.94)	1.12 (0.69–1.83)
DQB1*03	0.99 (0.67–1.46)	0.95 (0.64–1.42)
DRB1*13	1.14 (0.74–1.75)	1.09 (0.70–1.70)
DRB1*1501	1.13 (0.72–1.78)	1.01 (0.64–1.61)
DQB1*0602	1.13 (0.71–1.81)	0.98 (0.61–1.59)
DRB1*1501-DQB1*0602	1.13 (0.71–1.81)	0.98 (0.61–1.59)
B7-DRB1*1501	1.10 (0.59–2.04)	0.97 (0.51–1.82)
B7-DQB1*0602	1.07 (0.57–2.03)	0.94 (0.49–1.80)
B7-DRB1*1501-DQB1*0602	1.07 (0.57–2.03)	0.94 (0.49–1.80)
Positive for HPV16 or HPV18 (47 positive controls, 768 negative controls)		
B7	1.76 (0.89–3.47)	1.62 (0.81–3.26)
DQB1*03	0.89 (0.49–1.62)	0.82 (0.45–1.52)
DRB1*13	0.59 (0.27–1.29)	0.56 (0.25–1.24)
DRB1*1501	1.49 (0.77–2.87)	1.34 (0.68–2.65)
DQB1*0602	1.88 (0.98–3.59)	1.62 (0.83–3.18)
DRB1*1501-DQB1*0602	1.70 (0.88–3.29)	1.46 (0.74–2.89)
B7-DRB1*1501	2.70 (1.30–5.60)	2.45 (1.15–5.21)
B7-DQB1*0602	3.22 (1.58–6.58)	2.92 (1.39–6.13)
B7-DRB1*1501-DQB1*0602	2.87 (1.38–5.97)	2.59 (1.21–5.52)

[1]Adjusted for age and ethnicity.–[2]According to Munoz *et al*.[2] Includes HPV16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82 (including IS39).

in risk of cancer or HSIL was only reported for a single allele, HLA-CW*0202.

In contrast to many studies,[7,10–13] but similar to others,[15–17,19] the DQB1*03 allele group was not associated with increased risk of cervical neoplasia. Also, BCCR results pertaining to the DRB1*13 allele group are divergent from other studies (*e.g.*[12,15,19]) in that presence of the allele group was not protective against the development of HG-CIN.

In both the global and the oncogenic HPV-restricted analyses, presence of the B7-DRB1*1501-DQB1*0602 haplotype was protective against progression to HG-CIN. This effect was strong when the dataset was restricted to HPV 16/18-positive women with a 5-fold reduction in risk after adjustment for behavioral factors, age and ethnicity. The association became particularly robust when adjusted for the separate contributions of the B7 allele and DRB1*1501-DQB1*0602 haplotype. In the analysis of HPV 16/18 status among controls, ORs showed an opposite trend with evidence of an increased risk of HPV positivity associated with the presence of B7-DRB1*1501-DQB1*0602 haplotype, which remained elevated after adjustment for the contributing alleles,

HPV status in the control group is a cross-sectional prevalence measure, and thus HPV-positivity can be interpreted as an indirect estimation of HPV persistence conditional on age. Taken at face value, this analysis suggests that the mechanisms of mediation are likely to be more complex than simply *via* development of persistent infection. HLA could modify the course of infection or mediate the elimination of HPV-transformed cells through different mechanisms. Conceivably, an HLA haplotype could increase the risk of an oncogenic HPV infection while also leading to a more effective immune response leading to rapid viral clearance,[40] which would lower the risk of progression to high-grade CIN. This could be mediated *via* remote linkage with separate HLA gene loci influencing humoral and cell-mediated responses. These results must also be considered within the limitations imposed by a subset analysis of a case-control study and at best are hypothesis generating.

In conclusion, the B7-DRB1*1501-DQB1*0602 haplotype was moderately protective against development of HG-CIN in the BCCR Caucasian study population, and more strongly protective against risk of progression to HG-CIN in an analysis restricted to oncogenic-HPV positive subjects. This complex mediating effect seems to affect multiple endpoints in the natural history of HPV and CIN, as the same haplotype was associated with increased risk of HPV 16/18 infection among controls. In light of these findings and heterogeneity among published results for B7 and DRB1*1501-DQB1*0602, a plausible interpretation is that the B7-DRB1*1501-DQB1*0602 haplotype is in linkage disequilibrium with other unmeasured genetic polymorphisms in certain ethnic populations, presumably influential as etiologic cofactors in cervical neoplasia. Nongenetic factors associated with ethnicity can also be responsible for the variable results across studies. This issue could be remedied by pooling of typing data across studies. Because of ever-present concerns regarding false-positive associations as a result of multiple statistical testing, these putative associations must also be verified in cohort studies that are able to control confounding by ethnicity.

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