CONCISE COMMUNICATION

Human Leukocyte Antigen Class I and II Alleles and Risk of Cervical Neoplasia: Results from a Population-Based Study in Costa Rica

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To examine human leukocyte antigen (HLA) involvement in the development of all grades of cervical neoplasia, a nested case-control study of 10,077 women in Guanacaste, Costa Rica, was conducted. Participants had invasive cervical cancer, high-grade squamous intraepithelial lesions (HSILs; *n* = 166), or low-grade squamous intraepithelial lesions (LSILs); were positive for human papillomavirus (HPV) with no evidence of cervical neoplasia (*n* = 320); or were HPV negative with no evidence of cervical neoplasia but with a history of high-risk sexual behavior (*n* = 173). Compared with women who were HPV negative, women with HLA-DRB1*1301 were associated with decreased risk for cancer/HSILs (odds ratio [OR], 0.4; 95% confidence interval [CI], 0.2–0.7) and for LSILs/HPV (OR, 0.6; 95% CI, 0.3–0.9). Women with both HLA-B*07 and HLA-DQB1*0302 had an 8.2-fold increased risk for cancer/HSILs (95% CI, 1.8–37.2) and a 5.3-fold increased risk for LSILs/HPV (95% CI, 1.2–23.7). These results support the hypothesis that multiple risk alleles are needed in order to increase risk for cervical neoplasia, but a single protective allele may be sufficient for protection.

Numerous studies over the past decade have examined associations between HLA and cervical neoplasia [1–5]. HLA alleles, which are involved in presenting foreign antigens to immune cells, are important in host immune responses to viruses and other pathogens. Among the most polymorphic human genes, HLA polymorphisms result in variations of the peptide-binding cleft, influencing the antigens bound and presented to T cells. Class I HLA molecules (HLA-A, -B, and -C) present foreign antigens to $CD8^+$ cytotoxic T lymphocytes, and class II molecules (HLA-DR, -DQ, and -DP) present antigenic peptides to $CD4^+$ T helper cells. For cervical neoplasia, it is well established that human papillomavirus (HPV) is the etiologic agent. Therefore, given that host immune response to HPV is thought to be an important determinant of HPV acquisition and progression to high-grade lesions and cancer, it is plausible that HLA variations may affect cervical pathogenesis through immunologic control of HPV [6, 7].

Because epidemiologic studies to date have reported inconsistent associations between HLA and cervical neoplasia [1–5], we conducted 2 large independent case-control studies of cervical neoplasia in ethnically distinct populations. We sought to examine HLA involvement in the development of all grades of cervical neoplasia and to determine whether associations are homogeneous in 2 large and ethnically distinct populations. Results from our first case-control study—a nested study of a cohort of 24,000 women in Portland, Oregon, the majority of

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whom were white—have been reported elsewhere [8]. Here we present the results of our second case-control study, conducted among a 10,000-woman cohort in Guanacaste, Costa Rica.

Methods

Study population. This study was nested within an ongoing population-based cohort study of 10,077 women in Guanacaste, Costa Rica. Details of the cohort study methods have been reported elsewhere [9]. The present study was composed of 860 women: 36 diagnosed with invasive cancer; 130 with high-grade squamous intraepithelial lesions (HSILs); 105 with low-grade squamous intraepithelial lesions (LSILs) who tested positive for cancer-associated HPV types, by means of the first-generation Hybrid Capture Tube (HCT) test (Digene); 215 cytologically normal women positive for cancer-associated HPV types, by the HCT test; 173 cytologically normal women who were HPV negative, by the HCT test and polymerase chain reaction (PCR), but who reported having ⩾5 sex partners. The high-risk HPV-negative women are considered to have adequate immune protection against virus exposure. We also included 250 women randomly selected from the 10,077 cohort participants. (Of the 250, 49 were included in one of the study groups described above). Comparison of disease to the random sample provided a case-cohort approach; comparisons with HPV-negative women provided a case-control approach. We describe results from the case-control analyses, allowing for direct comparison with our US-based study results.

HPV testing. Study participants provided a Pap smear; samples initially were tested for HPV by use of the HCT test, as described elsewhere [10]. Subsequently, PCR-based HPV DNA testing that used L1 MY09/MY11 consensus primer methods was completed for 832 (96.7%) study participants [11].

HLA typing. DNA was extracted from buffy coats with PureGene purification kits (Gentra Systems). We developed an HLA-B*07 screen with single-stranded oligonucleotide primers (SSOPs): RC206 (XTAGGCGTACTGGTCATGC), RC194H (XGACAAGCTGGAGCGCGC), and consensus probe DB488 (XGGTCCAGGAGCTCAGGTCCTC) ("X" indicates horseradish peroxidase). RC206 detects HLA-B*0702, *0703, *0704, and *1510; RC194H detects HLA-B*0702, *0703, *0704, *0705, *4001, *4007, *4801, and *8101. Samples underwent PCR with 50 pmol RAP1021 (AACTGCAGCCTCCTCCGCGGG); RAP1022 (GGAGGCCATCCCCGGCGACCTAT); 1× buffer II; 1.5 m*M* $MgCl_2$; 200 μmol dATP, dCTP, and dGTP; 400 pmol dUTP; and 5 U AmpliTaq (Perkin-Elmer) in a Perkin-Elmer thermocycler (model TC9600; 40 cycles at 95°C, 30 s; 60°C, 45 s; 72°C, 1 min; and 10 min, 72°C). Then, 20 μL of denatured (1:2 dilution of 0.4 *N* NaOH) PCR product was dot blotted onto replicate nylon and immobilized (50 mJ; Stratalinker UV). SSOPs were hybridized to filters in 10 mL of hybridization buffer (3× saline sodium phosphate EDTA [SSPE] and 0.5% SDS; 2 pmol probe/mL hybridization buffer for 30 min) and washed (1× SSPE and 0.1% SDS for 15 min: 55°C for RC194 and RC206; 50°C for DB488). Membranes were rinsed (0.1 *M* sodium citrate, pH 5.0) and developed (50 mL of 0.1 *M* sodium citrate buffer, pH 5.0; 2.5 mL of 2.0 mg/mL 3,3′,5,5′-tetramethylbenzidine; and 25 μL of 30% H_2O_2 for 10 min). Samples positive for all 3 probes were considered to be B*07 positive. Because HLA-B*1510 frequency is very low (.005), it is unlikely that heterozygous genotypes involving *1510 and *4001, *4007, *4801, or *8101 affected our reported HLA-B*07 frequencies.

Samples were amplified, by PCR, for HLA-DRB1 and HLA-DQB1 and were probed with labeled SSOPs as described elsewhere [12, 13]. Group-specific amplifications for DRB1 subtyping (DR2–DR6 and DR8) were done as reported previously [14, 15]. Complete DRB1 and DQB1 typing was obtained successfully for 835 (97.4%) and 846 (98.4%) of the eligible subjects, respectively. Of 70 random samples blindly tested in duplicate, percent agreement for DRB1, DQB1, and B*07 was 92.9%, 93.6%, and 97.1%, respectively.

Statistical methods. HLA-DR, HLA-DQ, and HLA-B*07 allele frequencies were calculated, and statistical differences between study groups were identified by use of the χ^2 test for significance. Independent alleles and joint effects for which associations were demonstrated in our US-based study were assessed in this study (a priori alleles), as were alleles with significant differences between study groups in our present population (a posteriori alleles). We determined the magnitude and statistical significance of associations by means of odds ratios (ORs) and 95% confidence intervals (CIs). Although we used logistic regression to examine allele-disease associations while adjusting for other alleles (DRB*1301, DRB*1501, DQB1*0302, DQB1*0602, DQB1*0603, and B*07), adjustment did not alter results. Thus, we show unadjusted risk estimates and stratified results. Pearson correlation coefficients between these alleles were calculated to identify alleles potentially in linkage disequilibrium. Finally, because HPV-16 is the most common HPV type in cervical cancer, we also did HPV-16–restricted analyses.

Results

Final study groups. To assess risk for women with high- or low-grade disease, eligible persons were categorized into 3 groups: cancer/HSIL (n = 166), LSIL/HPV positive (n = 320), and HPV negative/⩾5 male partners (n = 173). The random sample (n = 250) served as a confirmatory control group. To ensure that HLA associations observed would not be obscured by the decision to combine the group with cancer with those with HSILs or to combine the group with LSILs with those who were cytologically normal and HPV positive, we compared HLA allele frequencies among these subgroups but found no notable differences. Allele-disease associations (by using HSILs and cancer as separate outcomes) also produced consistent results. Although attempts to distinguish between cervical intraepithelial neoplasia (CIN) 2 and CIN3 were hindered by small numbers, exclusion of CIN2 from the cancer/HSIL group also was consistent with the results presented.

HLA-disease association. A priori alleles consisted of those with associations observed in our US-based study: DRB1*1301, DRB1*1501, DQB1*0302, DQB1*0602, DQB1*0603, and B*07 (table 1). Significant allele-disease associations were observed for DRB1*1301 and DQB1*0603, both known to be in linkage disequilibrium. DRB1*1301 had a statistically significant decrease in risk in the cancer/HSIL group (OR, 0.4; 95%

Table 1. Independent associations between HLA-DRB1, -DQB1, and -B*07 alleles and disease status, compared with the allele status of human papillomavirus (HPV)–negative control subjects.

Allele present	Cancer/HSIL group (n = 166)			LSIL/HPV positive group (n = 320)			No. of HPV-negative control subjects (n = 173)
	No. of patients	OR	95% CI	No. of patients	OR	95% CI	
DRB1*1301							
Yes	12	0.4	0.2–0.7	35	0.6	0.3–0.9	31
No	153			284			142
DRB1*1501							
Yes	15	1.2	0.6–2.7	39	1.7	0.9–3.3	13
No	150			280			160
DQB1*0302							
Yes	75	1.3	0.8–2.0	158	1.5	1.0–2.2	70
No	86			155			102
DQB1*0602							
Yes	29	1.2	0.7–2.2	59	1.3	0.8–2.2	26
No	132			254			146
DQB1*0603							
Yes	12	0.4	0.2–0.8	40	0.7	0.4–1.3	28
No	149			273			144
B*07							
Yes	30	1.5	0.8–2.7	53	1.4	0.8–2.3	22
No	136			267			151

NOTE. Differences between the sum of the no. of patients and the total for each group *(n)* are due to missing HLA data. CI, confidence interval; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; OR, odds ratio.

CI, 0.2–0.7) and the LSIL/HPV positive group (OR, 0.6; 95% CI, 0.3–0.9). DQB1*0603 showed a statistically significant decrease in risk in the cancer/HSIL group (OR, 0.4; 95% CI, 0.2–0.8) and a nonsignificant decrease in risk in the LSIL/HPV positive group (OR, 0.7; 95% CI, 0.4–1.3). Decreases in risk in the cancer/HSIL group were more pronounced in the HPV-16–restricted analyses, in which DRB1*1301 and DQB1*0603 had ORs of 0.2 (95% CI, 0.1–0.7) and 0.1 (95% CI, 0.03–0.6), respectively. Although DQB1*0302 analyses showed a slight increase in risk in the cancer/HSIL group (OR, 1.3; 95% CI, 0.8–2.0) and the LSIL/HPV positive group (OR, 1.5; 95% CI, 1.0–2.2), these associations did not hold in the HPV-16–restricted analyses. No statistically significant risk increases for disease were observed for B*07. In the HPV-16–restricted analyses, there was a statistically significant increased risk only in the LSIL/HPV positive group (OR, 2.7; 95% CI, 1.2–5.7).

These allele-disease associations also were consistent with results for our case-cohort analyses, with a risk estimate of 0.6 observed for DRB1*1301 and the cancer/HSIL group and the converse estimate for HPV-negative women (OR, 1.5). Similarly, although negative associations were seen for HPV-negative women and DQB1*0603 or B*07, positive associations were observed with high-grade disease.

DRB1*1301, DQB1*0603, DRB1*1501, and DQB1*0602 are in linkage disequilibrium and were examined jointly (table 2). In our US-based study, the joint effect for B*07 and DQB1*0302 suggested excess risk of cervical neoplasia, which also was examined in this study. Linkage disequilibrium has not been reported for B*07 and DQB1*0302 and was not observed in our study (r = .1); moderate linkage disequilibrium was observed between DRB1*1501 and DQB1*0602 (r = .6) and for DRB1*1301 and DQB1*0603 (r = .7).

Women with both DRB1*1301 and DQB1*0603 had a decreased risk for cancer/HSILs (OR, 0.3; 95% CI, 0.1–0.7) and LSILs/HPV positivity (OR, 0.6; 95% CI, 0.4–1.2). In the HPV-16–restricted analyses, carrying both alleles resulted in a more pronounced decrease in risk in the cancer/HSIL group (OR, 0.1; 95% CI, 0.01–0.6) and the LSIL/HPV positive group (OR, 0.5; 95% CI, 0.2–1.6). Possession of DRB1*1301 and not DRB1*0603 resulted in ORs of 0.6 for the cancer/HSIL group and 0.4 for the LSIL/HPV positive group, neither of which was statistically significant. Possession of DQB1*0603 and not DRB1*1301 showed no associations with the cancer/HSIL group (OR, 0.9) or the LSIL/HPV positive group (OR, 1.1).

Possession of both B*07 and DQB1*0302 showed statistically significant increases in risk in the cancer/HSIL group (OR, 8.2; 95% CI, 1.8–37.2) and the LSIL/HPV positive group (OR, 5.3; 95% CI, 1.2–23.7). However, positivity for either B*07 or DQB1*0302 did not result in statistically significant or consistent risk increases. In the HPV-16–restricted analyses, those with B*07 and DQB1*0302 had an 8.0-fold increase in risk (95% CI, 1.6–40.7) for cancer/HSILs and a 7.3-fold increase in risk (95% CI, 1.1–47.2) for LSILs/HPV positivity. Unlike previous reports on B*07/DRB1*1501, neither linkage disequilibrium nor a joint effect was observed. Observed joint effects were confirmed with our case-cohort approach (e.g., an OR of 3.0 for B*07$^+$/DQB1*0302$^+$ and the cancer/HSIL group; conversely, an OR of 0.4 for B*07$^+$/DQB1*0302$^+$ and HPV-neg-

Table 2. Joint associations between HLA alleles and disease status, compared with the allele status of human papillomavirus (HPV)–negative control subjects.

Allele	r[a]	Cancer/HSIL group (n = 166)			LSIL/HPV positive group (n = 320)			No. of HPV-negative control subjects (n = 173)
		No. of patients	OR	95% CI	No. of patients	OR	95% CI	
DRB1*1301$^+$								
DQB1*0603$^+$	.7	7	0.3	0.1–0.7	29	0.6	0.4–1.2	23
DQB1*0603$^-$		5	0.6	0.2–1.8	6	0.4	0.1–1.1	8
DRB1*1301$^-$								
DQB1*0603$^+$		5	0.9	0.3–3.3	11	1.1	0.4–3.3	5
DQB1*0603$^-$		144	1.0	—	267	1.0	—	136
B*07$^+$								
DQB1*0302$^+$	.1	14	8.2	1.8–37.2	15	5.3	1.2–23.7	2
DQB1*0302$^-$		15	0.9	0.4–1.9	37	1.4	0.7–2.5	19
B*07$^-$								
DQB1*0302$^+$		61	1.0	0.7–1.7	143	1.5	1.0–2.2	68
DQB1*0302$^-$		71	1.0	—	118	1.0	—	83
DRB1*1501$^+$								
DQB1*0602$^+$	.6	12	1.2	0.5–2.9	30	1.6	0.8–3.3	11
DQB1*0602$^-$		3	1.7	0.3–10.2	9	2.6	0.6–12.4	2
DRB1*1501$^-$								
DQB1*0602$^+$		17	1.3	0.6–2.6	29	1.1	0.6–2.2	15
DQB1*0602$^-$		129	1.0	—	245	1.0	—	144

NOTE. Differences between the sum of the no. of patients and the total for each group *(n)* are due to missing HLA data. CI, confidence interval; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; OR, odds ratio.

[a] Pearson correlation coefficient between alleles.

ative women). Finally, while high-resolution genotyping for DRB1 and DQB1 allowed examination of a posteriori alleles, no additional consistent allele-disease associations were identified in our population. Details are given in the Appendix (published only in the electronic edition of the *Journal* [http://www.journals.uchicago.edu/JID/]). Tables A1 and A2 in the Appendix show the frequency distribution of class II DRB1 and DQB1 alleles by allele and study group.

Discussion

Our results confirm the reduction in disease risk with a single allele (DRB1*1301) and support the increase in risk from the presence of multiple risk alleles (B*07 and DQB1*0302). Others have reported protective associations between the presence of DRB1*13 alleles and the DRB1*1301/DQB1*0603 haplotype, for cervical cancer [1, 5, 8]. In our study, women possessing DRB1*1301 but not DQB1*0603 were at a consistently (although not statistically significant) reduced risk of disease. However, possessing DQB1*0603 but not DRB1*1301 did not reduce disease risk, suggesting that the protective effect seen with DRB1*1301/DQB1*0603 may be due to the DRB1 allele.

Although our results weakly suggested that B*07 and DQB1*0302 are associated individually with disease, despite the findings of previous studies [1–4], our stratified analysis demonstrated significant increases in disease risk when both alleles are present. These results support those from our US-based study [8], suggesting that the effect may be real and not due to chance or population stratification. The proportion of women possessing both alleles in our random sample was 3.2% and 8.7% in the cancer/HSIL group, suggesting a modest contribution to disease from these alleles.

We were unable to confirm the increase in disease risk for DRB1*1501/DQB1*0602, as reported previously, or the risk reduction seen in our US-based study [1, 3, 4, 8]. Although these discordant findings suggest the lack of a causal association in cervical neoplasia, we cannot ignore the possibility that differences seen in ethnically distinct populations are due to differences in linkage disequilibrium for these alleles.

Study strengths included the population-based design, high response rate, and extensive diagnostic workup that included 4 screening tests and multiple cytopathology reviews. In addition, our high-resolution HLA genotyping was much more extensive than that used in previous studies. The disease categories also provided a unique opportunity to evaluate consistency of associations across increasing severity of cervical cancer pathogenesis. The increasing magnitude of associations in the HPV-16–restricted analyses also confirmed expected HPV type specificity of HLA–cervical disease associations. Lastly, parallel analyses of allele-disease associations by using a random sample allowed internal validation of our findings. As expected, the HLA alleles that increased risk for cervical cancer were most common among women in the HSIL/cancer group and least common among cytologically normal, HPV-negative women (and vice versa). For risk alleles, this translated to a positive association with the HSIL/cancer group and a negative association with HPV-negative women; the opposite was observed for protective alleles.

Study limitations included initial reliance on the less sensitive first-generation HCT test; HPV-negative status was confirmed by PCR. Although small numbers of invasive cancers limited examination of HLA alleles associated with the high-grade to invasive disease transition, previous data suggest more similarities than differences between HLA alleles associated with HSILs and cancer.

In summary, results from our ethnically admixed Costa Rican population provide strong evidence for HLA involvement in progression to and protection from cervical neoplasia. A consistent pattern has emerged, suggesting that the presence of B*07 and DQB1*0302 increases risk for and DRB1*1301 protects against HPV infection and cervical disease. These findings support the hypothesis that multiple risk alleles are required to confer altered risk that is detectable at the population level, possibly through inadequate presentation of viral antigens to the immune system. In contrast, the presence of a single protective allele may be sufficient to confer protection.

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