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Comprehensive Analysis of HLA-A,-B, -C, -DRB1, and -DQB1 Loci and Squamous Cell Cervical Cancer Risk

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Abstract

Variation in human major histocompatibility genes may influence the risk of squamous cell cervical cancer (SCC) by altering the efficiency of the T cell mediated immune response to HPV antigens. We used high resolution methods to genotype HLA Class I (A, B, and Cw) and Class II (DRB1 and DQB1) loci in 544 women with SCC and 542 controls. Recognizing that HLA molecules are co-dominantly expressed, we focused on co-occurring alleles. Among 137 allele combinations present at >5% in the case or control groups, 36 were significantly associated with SCC risk. All but one of the 30 combinations that increased risk included DQB1*0301, and 23 included subsets of A*0201-B*4402-Cw*0501-DRB1*0401-DQB1*0301. Another combination, B*4402-DRB1*1101-DQB1*0301, conferred a strong risk of SCC (OR 10.0, 95% CI 3.0-33.3). Among the 6 combinations that conferred a decreased risk of SCC, 4 included Cw*0701 or DQB1*02. Most multilocus results were similar for SCC that contained HPV16; a notable exception was A*0101-B*0801-Cw*0701-DRB1*0301-DQB1*0201 and its subsets, which were associated with HPV16+ SCC (OR 0.5, 95% CI 0.3-0.9). The main multilocus associations were replicated in studies of cervical adenocarcinoma and vulvar cancer. These data confirm that T helper and cytotoxic T cell responses are both important cofactors with HPV in cervical cancer etiology, and indicate that co-occurring HLA alleles across loci appear to be more important than individual alleles. Thus, certain co-occurring alleles may be markers of disease risk that have clinical value as biomarkers for targeted screening or development of new therapies.

Keywords

Cervical Cancer; HLA; HPV; multilocus analysis; HLA class I and II polymorphisms

Introduction

Cervical cancer is the second most common cancer among women worldwide, with nearly 500,000 new cases and over 270,000 deaths occurring globally each year.(1) It is primarily caused by a common sexually transmitted pathogen, oncogenic human papillomavirus (HPV). HPV has been shown to meet the causal criteria for cervical cancer, including viral presence in tumors, consistent findings of strong associations across a variety of laboratory-based and epidemiologic studies, a viral dose-response relationship, a pathologic sequence in which infection precedes development of lesions, and mechanistic coherence.(2) Since HPV is a common infection in young women and cancer is a rare outcome, other factors such as individual immune response to the infection are likely necessary to promote persistent HPV infection and the development of increasingly severe cervical intraepithelial neoplasia that precedes invasive cervical cancer.

The human leucocyte antigen (HLA) class I and II molecules play a critical role in the manner that HPV peptides are presented to T cells. High-affinity engagement of a T cell receptor with an HPV peptide-HLA complex and a costimulatory signal is necessary to activate a T cell response, and may vary with HLA type.(3) Class I HLA molecules are found on most nucleated cells and present peptides from the cytosol to cytotoxic T cells; on the other hand, Class II HLA molecules are found on antigen-presenting cells (e.g., dendritic cells and macrophages) and present peptides degraded in intracellular vesicles to helper T cells.(4) An effective immune response may require optimal peptide presentation by both class I and class II molecules in order to activate efficient helper and effector T cell responses to HPV. Subtle changes or impairment in T cell responses may allow escape from immune surveillance or induction of immune anergy or tolerance to HPV peptides.

In this study we conducted high-resolution HLA typing to assess the risk of squamous cell cervical cancer (SCC) associated with class I HLA-A, -B, and -C and class II HLA-DRB1 and -DQB1 loci. HLA loci are extraordinarily polymorphic, so few haplotypes are present at high frequency across all five loci and hence typical haplotype-based association analysis is not warranted. Recognizing that HLA molecules are co-dominantly expressed on the cell surface (i.e., both alleles at each locus are expressed in heterozygous individuals) and that combinations of alleles at various HLA loci are jointly informative, we focused the present analysis on co-occurring alleles across HLA loci.

Methods

Study Population

Cases were 18-74 year old women diagnosed with invasive squamous cell cancer (SCC) while residents of the 3-county metropolitan Seattle area (King, Snohomish, and Pierce Counties) in Washington State in two time periods, January 1986 through June 1998 or January 2000 through December 2004. Cases were ascertained from a National Cancer Institute cancer registry based in Seattle (the Cancer Surveillance System, a Surveillance and End Results (SEER) registry), and had been assigned International Classification of Diseases for Oncology morphology codes 8010-8077 and topography codes 1800-1809. Controls were identified through random-digit telephone dialing (RDD) among female residents of the same counties, and were frequency matched to the age distribution of the cases in 5-year groups. Eligible controls for the cervical cancer study had to have an intact uterus. Both case and control subjects in this study had to have a residential telephone and the ability to communicate in English.

In-person interviews covered demographic characteristics as well as reproductive, sexual, birth control, hormonal, Pap, and smoking histories. Previous reports from this study have examined a variety of risk factors for cervical cancer including HPV status,(5) oral contraceptive use,

cigarette smoking, and herpes simplex virus,(6) the p53 Arg72Pro polymorphism,(7) *Chlamydia trachomatis*,(8) and HLA class II alleles.(9) Questions on the interview referred to events prior to the diagnosis date (month and year) for cases or an assigned date in the past for controls. Control reference dates were matched to the distribution of diagnosis dates of the cases.

The response proportion was 62.6% for cases (744 interviewed out of 1189 cases) and 66.5% for controls (1486 interviewed out of 2024 controls). The response proportion for controls accounts for the household screening response rate (90.6%, the proportion of all potential controls who were screened and found to be eligible), according to our protocol for RDD control selection.(10) Collection of samples suitable for DNA extraction started several years after interviewing began for the study. Although all case subjects with DNA available were included in this study (544 of 744 interviewed cases), controls were sampled to match the number of cases in the study. A total of 544 women with SCC and 542 control women had samples available for HLA testing at all 5 loci and are included in this analysis.

HLA Genotyping

Peripheral blood samples were collected in Vacutainer tubes containing EDTA anticoagulant and processed and stored at -80°C within 24 hours of being drawn. DNA was extracted from stored peripheral blood mononuclear cells that had been suspended in RPMI 1640/DMSO using a manual phenol:chloroform method, and stored in an ultra-low freezer in multiple 0.5 ml aliquot tubes. Two methods were used to type HLA class I and II alleles, both of which used sequence specific oligonucleotide probe (SSOP) reverse format assays (rSSOP). Initially, the Dynal RELI™ system with probe arrays bound to nylon membrane strips was employed. Later typing used the One Lambda LabType™ system with probe arrays bound to color coded plastic microspheres was used. In both methods, locus-specific biotinylated primers for HLA DRB1 and DQB1 loci were used to amplify all known alleles for exon 2, while locus-specific primers for HLA A, B, and Cw were used to amplify all known alleles for exons 2 and 3. Biotinylated amplicons were denatured to single stranded DNA and incubated with individual line strips (RELI™) or tubes of pooled microspheres-probes (LabType™). Each of the five HLA loci was separately amplified and hybridized with locus specific probe arrays. The biotin-labeled amplicons hybridized to those SSOPs that contain a target DNA sequence complementary to the sample DNA. After hybridization, a stringent wash removed excess, unbound amplicon to ensure specificity. In the RELI™ system, visualization of the amplicon-probe complex consisted of two steps: (a) streptavidin-horseradish peroxidase (SA-HRP) conjugate bound the biotin-labeled amplicons captured by the SSO probes and (b) hydrogen peroxide and tetramethylbenzidine substrate formed a blue-colored complex with the SA-HRP conjugate. A transparent overlay with numbered lines corresponding to probe locations was placed over each individual membrane strip. Probe reactions were scored positive or negative with reference to the positive control. These results were reviewed subject to a second review to ensure accuracy. In the LabType™ system, SSOP reactions were analyzed with Luminex™ Flow Analysis equipment, in which the locus specific SSOP-bead arrays are subjected to laser interrogation to identify each specific probe and determine the presence (positive hybridization) or absence (negative hybridization) of the biotin-labeled amplicon. SSOP reactions for each sample are submitted electronically to an HLA analysis program to deduce the HLA type.

The Dynal RELI™ rSSOP method provided sufficient resolution of HLA A, B, C, and DQB1 alleles for our analyses. DRB1 alleles were specifically identified by DNA sequencing, with separate amplification of the polymorphic DRB1 allele families (DRB1*01, *02, *04, *03/11/13/14, *08/12) based on the preliminary rSSOP RELI™ analysis. DNA sequencing used fluorescence-labeled dideoxynucleotide terminator chemistry with analysis on an ABI

377-XL96 DNA sequencer using ABI Sequence Navigator and HLA Matchmaker software (Applied Biosystems). The One Lambda LabType™ rSSOP method employed a sufficient number of probes to assign alleles for our analysis at all HLA loci, although infrequent or rare HLA alleles identified by LabType™ were confirmed by DNA sequencing using fluorescence-labeled dideoxynucleotide terminator chemistry (Atria Genetics) with analysis on an ABI 3130xl sequencer with Assign™ HLA analysis software (Conexio Genomics).

For DQB1*02, the difference between DQB1*0201 and *0202 is in exon 3, and the typing reagents we use analyze polymorphisms in exon 2. Therefore, specific allele assignments were made based on common allele specificities in the presence of DRB1*0301 or DRB1*0701. (11) Thus, DQB1*02 became DQB1*0201 if a subject carried DRB1*0301 and DQB1*0202 if a subject carried DRB1*0701. If a person was typed with DQB1*02 and had neither DRB1*0301 nor *0701, then the DQB1*02 allele could not be inferred, and was kept as missing for both allele-level variables.

HPV Typing of Tumor Tissue

Tissue blocks from 389 of 544 case subjects (71.5%) were retrieved from local pathology laboratories, and HPV typing was performed using PCR/RFLP (MY09/MY11 L1 consensus and HPV16 and HPV18 E6 type-specific primers, with co-amplification of 236 bp or 536 bp fragments of β -globin to test for sample integrity). While this would not be the optimal testing method by current standards, our intention was to use the optimal method available at the start of the study and continue with a standard approach throughout the study. Newer assays would likely have found additional positive samples as well as additional HPV types. Human β -globin DNA was detected in 369 (94.9%) of the tumors and HPV DNA was found and typed in 332 of those tumors (90.0%).

Single Locus Analysis

Alleles present in 5% or more of the case or control subjects were included in the main single locus analyses. Odds ratios (ORs) and 95% confidence intervals (CIs) for the association between individual alleles within a locus and SCC risk were estimated by logistic regression, with adjustment for race (white, non-white). Additional analyses were restricted to whites only, but were similar to the race-adjusted analyses and are not presented here.

The Holm procedure was used to assess the likelihood that significant allele estimates were due to multiple comparisons.(12) This procedure is similar to the Bonferroni method in assessing the potential for type 1 error but with the added benefit of sequentially ordered Wald p values to rank the severity of the penalty for multiple comparisons.(13) Holm p-values are presented for the statistically significant odds ratios, and were calculated based on all tests conducted for each locus in Tables 2 and 3, for all results presented in Table 4, and for all results within a cancer site for Table 5. Information on individual alleles found less commonly in the study population, (i.e., at <5%), is presented in a supplementary Table (Supplementary Table 1) for comparison with other study populations.

Multilocus Analysis

To focus the analysis with meaningful sample sizes, we examined the co-occurring alleles that occurred in more than 5% of the case or control population. We used publicly available software (HPlus) to estimate the most commonly cooccurring alleles (<http://qge.fhrc.org/hplus>). Separate categorical variables were constructed to identify those unphased genotypes that include a particular allelic combination versus those unphased genotypes that can never give rise to the allelic combination, and we then used logistic regression to assess its association with SCC. Co-occurring alleles identified by HPlus that were present in 5% or more of the

cases or controls but not significantly associated with SCC are presented in a supplement to this report (Supplementary Table 2).

In a separate analysis of constituent alleles, we compared mutually exclusive multilocus combinations of interest to a common reference group in an attempt to determine the relative importance of single alleles compared to co-occurring alleles. To limit the number of comparisons in these analyses, only the four multilocus combinations of most interest were explored.

Replication of Findings in Other HPV-Related Anogenital Cancers

To assess whether the multilocus alleles found to be associated with cervical SCC are 1) not false positives, and 2) indicative of an effect of HLA molecules on the pathogenesis of HPV-related anogenital cancer more generally, we examined the association of these alleles with cervical adenocarcinoma(14) and vulvar carcinoma(15) using cases from our ongoing studies of those cancers. In brief, there were HLA results available from 424 squamous cell vulvar cancer cases and 501 adenocarcinomas of the cervix, with 66.7% and 68.7% response proportions, respectively. A single control group was used for all three case populations, with the addition of controls with a prior hysterectomy (n=134) to our vulvar cancer study.

Results

Consistent with other studies of cervical cancer, women with SCC in this study had more sexual partners compared with women without cervical cancer and the cases were more frequently current smokers compared to controls (Table 1). Case and control subjects in this study were predominately white by self report, reflecting the racial composition of the Seattle area. Among non-whites, more cases than controls identified themselves as Asian or Pacific Islanders.

Race adjusted odds ratios for single locus HLA Class I and II alleles present among at least 5% of the case or control subjects are presented in Tables 2 and 3. High-resolution DNA-based assay methods resulted in typing 40 A, 74 B, 32 Cw, 45 DRB1, and 17 DQB1 alleles. We found significantly elevated risks of SCC associated with three class I alleles (Table 2) that were present in over 20% of the cases: A*0301 (OR 1.4, 95% CI 1.1-1.9), B*4402 (OR 1.9, 95% CI 1.4-2.7), and Cw*0501 (OR 1.6, 95% CI 1.2-2.3). B*1501 was associated with a decreased risk of SCC (OR 0.6, 95% CI 0.4-0.9), and was found in 6.3% of cases and in 11.3% of the control subjects.

Two class II alleles (Table 3) were found to confer increased risk associated with SCC, i.e., DRB1*1101 (OR 2.1, 95% CI 1.3-3.2) and DQB1*0301 (OR 1.5, 95% CI 1.1-1.9). Decreased risks of SCC were associated with DRB1*1302 (OR 0.5, 95% CI 0.3-0.9) and DQB1*02 (OR 0.7, 95% CI 0.6-1.0) which were present among 9.1% and 41.8% of controls, respectively.

In exploratory analyses, co-occurring alleles comprising 2 to 5 loci present in more than 5% of the cases or controls were analyzed to comprehensively explore the potential joint impact of HLA class I and II genes. There were 137 co-occurring allele combinations that occurred in >5% of the cases or controls. As shown in Table 4, 36 co-occurring allele combinations conferred a significant risk of SCC: 30 were associated with increased risk and 6 associated with decreased risk.

The majority of the extended co-occurring alleles were associated with similar risks as lower-order sub-combinations. For example, a 2-fold increased risk of cervical cancer associated with the 5-allele combination A*0201-B*4402-Cw*0501-DRB1*0401-DQB1*0301, might be explained by the presence of the 4-allele combination B*4402-Cw*0501-DRB1*0401-

DQB1*0301, the 3-allele combination B*4402-DRB1*0401-DQB1*0301, a number of 2 allele combinations, or even by the individual alleles B*4402, DRB1*0401, or DQB1*0301.

The most commonly occurring allele in cases and controls was A*0201; however, this gene was not associated with SCC by itself, suggesting that it was passively associated with disease risk secondary to significant disequilibrium with alleles that affect risk. Interestingly, another common allele, DQB1*0301, was present in all but one of the 30 significant increased risks associated with co-occurring alleles (Table 4). The sole exception was A*0301-DQB1*0501. However, the risk of SCC associated with the A*0301-DQB1*0501 combination may have been due to the higher prevalence of DQB1*0301 among cases (28.6%) than controls (7.1%) who carried the A*0301-DQB1*0501 alleles (data not shown).

In Table 4, the most strongly increased risks were associated with B*4402-DRB1*1101 or B*4402-DRB1*1101-DQB1*0301 (OR 10.5, 95% CI 3.2-34.8 and OR 10.0, 95% CI 3.0-33.3, respectively). The elevated B*4402-DRB1*1101 estimate is a departure from the expected joint effect of these alleles (p for interaction=0.003).

There were fewer multilocus combinations that conferred a decreased risk of SCC than increased risk. Among the co-occurring alleles that conferred decreased risk, over 20% of the controls had Cw*0701 or DQB1*02, which were found in four of the six reduced-risk combinations. There were also decreased risks associated with A*0201-B*1501 (OR 0.4, 95% CI 0.2-0.7) and A*1101-Cw*0401 (OR 0.5, 95% CI 0.3-1.0).

In the leftmost odds ratio estimates in Table 5, we further explored multilocus associations with SCC by using a common reference group for each analysis. Taking this approach, the risks for various co-occurring allele combinations were compared to subjects without the alleles of interest. For B*4402-DRB1*1101-DQB1*0301 and B*0702-Cw*0702-DQB1*0301, the elevated risks depend on the multilocus allele combinations, not on individual alleles. Similarly, risk-detering DRB1*0301-DQB1*0201 related estimates (with or without B*0801) are lower than estimates for any constituent alleles (i.e., DRB1*0301 or DQB1*0201).

In the Seattle population, B*0702 was in tight linkage with Cw*0702, and the combination of these alleles with DQB1*0301 resulted in a strongly elevated risk of SCC (OR 2.7, 95% CI 1.6-4.6). When we extended this multilocus combination to either include or exclude DRB1*1501-DQB1*0602, (which was not by itself associated with SCC risk), both strata resulted in similarly increased risks of SCC (B*0702-Cw*0702-DQB1*0301 with DRB1*1501-DQB1*0602, OR 2.1, 95% CI 1.0-4.3 and without DRB1*1501-DQB1*0602, OR 3.3, 95% CI 1.6-6.8), suggesting no effect of DRB1*1501-DQB1*0602 in this population.

One association of interest in other studies involves DRB1*13-DQB1*06. The strongest risk estimates associated with SCC were for the DRB1*13-DQB1*06 allele combination (0.6, 95% CI 0.3-1.0). The analysis in Table 5 suggests that neither DRB1*13 or DQB1*06 alone is responsible for the decreased risk of SCC, but both are important together. Surprisingly, it appears that DRB1*13-DQB1*0301/06 does not confer a decreased risk of SCC (OR 1.1, 95% CI 0.6-1.8), suggesting that the combination of risk conferring (e.g., DQB1*0301) and risk decreasing alleles may result in a neutral effect.

The risk estimates for single alleles associated with SCC were similar when the case group was restricted to HPV16 positive cases (HPV16+SCC, n=228) for most of the single locus analyses presented in Tables 2 and 3. When the multilocus analyses were restricted to tumors that contained HPV16, most risk estimates were similar to those presented in Table 4 for all SCC and are presented in Supplementary Table 3. Strikingly, the allele combinations associated with a decreased risk of HPV16+ are represented by the 5 allele combination A*0101-B*0801-

Cw*0701-DRB1*0301-DQB1*0201 (OR 0.5, 95% CI 0.3-0.9), and the other HPV16-associated risk-decreasing allele combinations are subsets of that combination.

Table 5 presents the results of our attempt to replicate allele combination findings for cervical SCC using cases of cervical adenocarcinoma (n=537) and squamous cell vulvar carcinoma (n=424) from our studies, compared to a shared control group. There was an increased risk, but not as strongly increased risk associated with B*4402-DRB1*1101-DQB1*0301 in the adenocarcinoma of the cervix and vulvar studies. Also, there were increased risks associated with B*0702-Cw*0702-DQB1*0301 for all three case groups. There was a suggestion that the strong decrease in SCC risk associated with DRB1*0301-DQB1*0201 seemed to be more important for adenocarcinoma and vulvar cancer when B*0801 was also present as part of the allele combination.

DRB1*13-DQB1*06 was associated with decreased risk for all three case groups, but there was not an increased risk for cancer associated with DRB1*13-DQB1*06 in the presence of DQB1*0301.

Discussion

We report associations for extended HLA class I and II multilocus alleles that to our knowledge have not been previously reported, such as an increased risk associated with the multilocus B*4402-Cw*0501-DRB1*0401-DQB1*0301 (OR 2.3, 95% CI 1.3-3.7) and decreased risks associated with allele combinations containing only class I alleles, such as A*0201-B*1501 (OR 0.4, 95% CI 0.2-0.7) and B*0801-Cw*0701 (OR 0.7, 95% CI 0.5-0.9). In this study, B*4402 was found to be a major risk-conferring allele that appears often among cases with the known risk allele DQB1*0301. A prior study(16) reported an increased risk of progression to neoplasia in 8 out of 88 HPV16 positive women, 6 of whom were B*4402 carriers. In the current study, carriage of B*4402-DRB1*1101-DQB1*0301 was associated with an 11-fold increased risk of SCC (OR 11.5, 95% CI 3.5-38.5 in Table 5). This joint effect was reproduced at lower magnitude in two additional case groups of HPV-related anogenital cancer: we found a nearly 5-fold increased risk of adenocarcinoma of the cervix (OR 4.9, 95% CI 1.4-17.2) and vulvar cancer (OR 4.7, 95% CI 2.0-10.7) associated with these cooccurring alleles. Replication of this association and other multilocus combinations in two independent case groups from our anogenital cancer studies suggest that specific cooccurring HLA alleles may be more important than individual alleles.

In this population-based study, there were four HLA class I and four class II alleles significantly associated with cervical SCC after correction for multiple comparisons. One allele from each locus was associated with an elevated risk of SCC: A*0301, B*4402, Cw*0501, DRB1*1101, and DQB1*0301. Three alleles were associated with a decreased risk of SCC: B*1501, DRB1*1302, and DQB1*02. As can be seen in Table 4, risk conferring alleles tend to occur together. For example, the 5-loci combination A*0201-B*4402-Cw*0501-DRB1*0401-DQB1*0301 was associated with a 2-fold elevated risk of SCC, and contained three individually significant alleles (B*4402, Cw*0501, and DQB1*0301).

Previous work from our group(9) and others (17,18), indicates that risk of cervical cancer may depend on specific HLA alleles or HLA-linked genes. As genotyping of the HLA region has become more precise, observations of associations between HLA and cervical cancer have also become more refined. Consistent findings of increased risk of cervical cancer associated with DQB1*0301 and decreased risk associated with DRB1*13 have been reported from most populations throughout the world. However, less consistent results for other HLA alleles have been reported as well, and may represent population specific or even chance findings because of the polymorphic nature of the HLA region genes.

Several recent studies have examined the risk of cervical neoplasia associated with the HLA region. Zoodsma et al., (19) in a study conducted in the Netherlands, used microsatellite markers and two single nucleotide markers (SNPs) on 6p21 and found two markers (one near the HLA DQ and DR genes, the other in MICA) that were strongly associated with risk of cervical cancer. Engelmark et al. (20) used an affected sib pair approach in a Swedish study that performed typing of the five class I and class II loci, and reported a strong effect of class II but not class I loci in cervical cancer. A study by Hildesheim et al. (21) examined the risk of cervical neoplasia associated with one class I allele (B*07), and four class II allele families (DQB1*03, DQB1*06, DRB1*15, and DRB1*13) in a nested case-control study in Portland, Oregon. They reported increased risks of cervical neoplasia associated with B*07 and DQB1*0302 and decreased risk with DRB1*13. Wang et al., (22) in a study nested within the Guanacaste, Costa Rica, cohort, also reported strong risks associated with carriage of these five allele families for cervical neoplasia. A more recent study (23) from this group of investigators found no increased risks for cervical neoplasia associated with cervical cancer in a haplotype-based analysis, with high resolution typing across A-B-DR alleles in studies conducted in the Eastern US and Costa Rica.

In a UK study, (24) HLA-B7-positive cervical cancer patients had a significantly poorer clinical outcome than HLA-B7-negative patients. A significant component of the effect was down-regulation of HLA-B7 expression. In an earlier study, the same group (25) reported that an HPV16E6 non-wild type variant was present in 7 of 22 (31.8%) tumors from B7-positive cervical cancer patients. They reported that this variant alters the B7 binding epitope in a way that may influence cytotoxic T cell recognition. We did not have information on HPV16 viral variants in the present study, so a joint effect of B*07 and the HPV16E6 non-wild type variant could have been masked. Even without information on viral variants, however, the analysis in Table 5 suggests that co-occurrence of B*07 with two other alleles (i.e., B*0702-Cw*0702-DQB1*0301, OR 2.8, 95% CI 1.7-4.5), is important to increased risk of SCC.

DRB1*13 carriers may also be at decreased risk for other infectious diseases. HIV positive patients with DRB1*13-DQB1*06 had slower progression to AIDS and were better suppressors of viral activity than patients with other HLA haplotypes. (26). In another study, DRB1*1301 (but not 1302) was associated with decreased risks of malaria and hepatitis B. (27) These findings suggest that DRB1*13 may be especially good at presenting viral or tumor associated peptides to class II restricted T helper cells and promoting an effective immune response. Our study indicates that both DRB1*1301 and DRB1*1302 may be important in decreasing cervical cancer risk, but the most strongly decreased risk is associated with DRB1*1302. This difference may be due to the one amino acid difference between the two alleles, as DRB1*1302 has a G at the anchor position 86 in the peptide binding site compared to a V for DRB1*1301.

Limitations of this study need to be acknowledged. First, the highly polymorphic nature of HLA alleles leads to multiple comparisons and increases the risk of chance associations. We used the Holm's test, a Bonferroni-like method, to estimate the likelihood of a type 1 error for the single locus analyses. We also repeated the main exploratory analyses in two other case groups from our series of case-control studies of anogenital cancer in the Seattle area: women with adenocarcinoma of the cervix and women with vulvar cancer. It will be important to repeat these analyses in other populations and with other HPV-related end points to understand the reach of these findings; however, replication of the main exploratory findings in independent samples in the present study decreases the likelihood of chance findings due to multiple comparisons.

Second, the relatively low proportion of eligible cases (62.6%) and controls (66.5%) that enrolled in this study could potentially hamper interpretation of the results if, differentially

between cases and controls, carriers of certain alleles are over- or under-represented among those individuals who did not participate. It is reassuring, however, that the frequency of major HLA alleles among controls in our study is consistent with findings from other Caucasian populations.(28) For all subjects, it is not likely that a decision to participate in a research study is influenced by genetic factors. It is plausible, however, that certain HLA alleles could be over- or under-represented in our cases due to an impact on survival. If certain HLA alleles are associated with rapid progression of disease and poorer survival, these alleles may be under-represented in our case group, since 10.4% of SCC cases died prior to study enrollment.

This study is the first to report that commonly co-occurring class I and class II HLA alleles across loci may have a significant impact on cervical cancer development. They suggest that class II restricted CD4+ T helper as well as class I restricted CD8+ T effector cell recognition are necessary for a protective immune response to HPV-specific antigens or tumor antigens that may be unrelated to HPV. In this study, the presence of DQB1*0301 was found in most risk conferring allele combinations, and seemed to offset the reduced risk associated with DRB1*13 allele combinations if both DRB1*13 and DQB1*0301 were present.

The similarities between results from our squamous cell cervical cancer, vulvar, and adenocarcinoma of the cervix studies support the view that the HLA associations reported in this study are likely HPV-specific. If these findings can be repeated in other studies with different populations, they warrant further investigation. The use of a prophylactic vaccine to stimulate immune response to initial HPV infections indicates that continued examination of immune response to HPV may lead to the development of novel therapies or effective vaccines against established HPV infections.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1
Distribution of age, race, cigarette smoking, and lifetime number of sex partners among SCC cases and controls tested for HLA class I and II alleles

	Controls (N=542)		Cases (N=544)	
	n	%	n	%
Age	18-29	76	60	(11.1)
	30-39	175	180	(33.1)
	40-49	151	151	(27.8)
	50-59	68	83	(15.3)
	60-69	43	56	(10.3)
	70-74	29	14	(2.6)
Race	White	513	481	(88.4)
	Black	13	15	(2.8)
	American Indian	0	6	(1.1)
	Asian/Pacific Islander	10	25	(4.6)
	Mixed	6	17	(3.1)
Smoking	Never	262	203	(37.3)
	Former	161	143	(26.3)
	Current	119	198	(36.4)
Sex Partners	1	131	54	(9.9)
	2 to 4	149	168	(30.9)
	5 to 14	195	229	(42.2)
	15+	62	92	(16.9)

Table 2
Risk of Squamous Cell Cervical Cancer Associated with HLA class I A, B, and Cw alleles

	Controls (N=504)		Cases (n=490)		OR [†]	95% CI	Helm p-value ²
	n	%	n	%			
A*0101	154	(30.6)	143	(29.2)	1.0	(0.8-1.3)	
A*0201	249	(49.4)	213	(43.5)	0.8	(0.6-1.1)	
A*0301	120	(23.8)	143	(29.2)	1.4	(1.1-1.9)	0.02
A*1101	60	(11.9)	59	(12.0)	1.0	(0.7-1.5)	
A*2402	76	(15.1)	90	(18.4)	1.2	(0.8-1.7)	
A*2902	40	(7.9)	27	(5.5)	0.7	(0.4-1.1)	
A*3201	31	(6.2)	40	(8.2)	1.5	(0.9-2.4)	
A*6801	28	(5.6)	25	(5.1)	0.9	(0.5-1.6)	
B*0702	127	(25.2)	140	(28.6)	1.2	(0.9-1.6)	
B*0801	124	(24.6)	89	(18.2)	0.7	(0.5-1.0)	0.15
B*1501	57	(11.3)	31	(6.3)	0.6	(0.4-0.9)	0.02
B*1801	38	(7.5)	29	(5.9)	0.8	(0.5-1.3)	
B*2705	43	(8.5)	28	(5.7)	0.7	(0.4-1.1)	
B*3501	54	(10.7)	46	(9.4)	0.8	(0.5-1.3)	
B*4001	58	(11.5)	61	(12.4)	1.1	(0.8-1.7)	
B*4402	70	(13.9)	112	(22.9)	1.9	(1.4-2.7)	0.0001
B*4403	49	(9.7)	44	(9.0)	0.9	(0.6-1.4)	
B*5101	48	(9.5)	53	(10.8)	1.1	(0.7-1.7)	
B*5701	38	(7.5)	37	(7.6)	1.1	(0.7-1.7)	
Cw*0102	34	(6.7)	37	(7.6)	1.0	(0.6-1.7)	
Cw*0202	46	(9.1)	32	(6.5)	0.7	(0.4-1.2)	
Cw*0303	43	(8.5)	42	(8.6)	1.1	(0.7-1.7)	
Cw*0304	83	(16.5)	74	(15.1)	0.9	(0.7-1.3)	
Cw*0401	92	(18.3)	82	(16.7)	0.9	(0.6-1.2)	
Cw*0501	72	(14.3)	100	(20.4)	1.6	(1.2-2.3)	0.005
Cw*0602	94	(18.7)	85	(17.3)	1.0	(0.7-1.3)	
Cw*0701	152	(30.2)	116	(23.7)	0.8	(0.6-1.0)	

	Controls (N=504)		Cases (n=490)		OR ¹	95% CI	Holm p-value ²
	n	%	n	%			
Cw*0702	139	(27.6)	152	(31.0)	1.2	(0.9-1.5)	
Cw*0704	13	(2.6)	26	(5.3)	2.1	(1.0-4.1)	0.07
Cw*0802	34	(6.7)	30	(6.1)	0.9	(0.6-1.5)	
Cw*1203	34	(6.7)	23	(4.7)	0.7	(0.4-1.2)	
Cw*1502	16	(3.2)	27	(5.5)	1.6	(0.8-3.0)	
Cw*1601	42	(8.3)	30	(6.1)	0.7	(0.4-1.1)	

¹ Odds ratios are adjusted for race. Bolding represents significant associations, although rounding sometime caused confidence intervals to include 1.0.

² The Holm p value corrects the Wald p value for multiple comparisons and was based on all alleles at a locus and is only shown for alleles with significant odds ratios.

Table 3

Risk of Squamous Cell Cervical Cancer Associated with HLA class II DRB1 and DQB1 alleles

	Controls (N=538)		Cases (n=538)		OR ¹	95% CI	Holm P value ²
	n	%	n	%			
DRB1*0101	79	(14.7)	80	(14.9)	1.1	(0.8-1.5)	
DRB1*0301	135	(25.3)	100	(18.6)	0.7	(0.5-1.0)	0.08
DRB1*0401	100	(18.6)	121	(22.5)	1.4	(1.0-1.8)	0.22
DRB1*0404	48	(8.9)	39	(7.2)	0.8	(0.5-1.3)	
DRB1*0701	139	(25.8)	132	(24.5)	1.0	(0.7-1.3)	
DRB1*0801	30	(5.6)	25	(4.6)	0.9	(0.5-1.5)	
DRB1*1101	33	(6.1)	65	(12.1)	2.1	(1.3-3.2)	0.001
DRB1*1301	59	(11.0)	40	(7.4)	0.7	(0.4-1.0)	
DRB1*1302	49	(9.1)	28	(5.2)	0.5	(0.3-0.9)	0.03
DRB1*1401	25	(4.6)	28	(5.2)	1.1	(0.6-1.9)	
DRB1*1501	143	(26.6)	147	(27.3)	1.1	(0.8-1.4)	
DQB1*02	225	(41.8)	183	(34.0)	0.7	(0.6-1.0)	0.04
DQB1*0201 ³	135	(25.1)	100	(18.6)	0.7	(0.5-1.0)	0.08
DQB1*0202 ³	101	(18.8)	87	(16.2)	0.9	(0.6-1.2)	
DQB1*0301	169	(31.4)	216	(40.1)	1.5	(1.1-1.9)	0.003
DQB1*0302	119	(22.1)	111	(20.6)	0.9	(0.7-1.2)	
DQB1*0303	55	(10.2)	64	(11.9)	1.2	(0.8-1.7)	
DQB1*0402	23	(4.3)	28	(5.2)	1.1	(0.6-1.9)	
DQB1*0501	100	(18.6)	106	(19.7)	1.1	(0.8-1.5)	
DQB1*0503	23	(4.3)	28	(5.2)	1.2	(0.7-2.1)	
DQB1*0602	146	(27.1)	147	(27.3)	1.0	(0.8-1.4)	
DQB1*0603	61	(11.3)	43	(8.0)	0.7	(0.5-1.0)	
DQB1*0604	35	(6.5)	21	(3.9)	0.6	(0.3-1.0)	

¹ Odds ratios are adjusted for race. Bolding represents significant associations, although rounding sometimes caused confidence intervals to include 1.0.² The Holm p value corrects the Wald p value for multiple comparisons and was based on all alleles at a locus and is only shown for alleles with significant odds ratios.

³ DQB1*02 could not be discerned as *0201 versus *0202 by the assay; see the Methods section.

Table 4
Significant risks of SCC associated with class I and II multilocus genotypes

Loci	Alleles	Controls (N=502)		Cases (N=490)		OR [†]	95% CI
		n	%	n	%		
A-B-Cw-DRB1-DQB1	0201-4402-0501-0401-0301	18	(3.6)	32	(6.5)	2.0	(1.1-3.7)
A-B-Cw-DRB1	0201-4402-0501-0401-xxxx	20	(4.0)	36	(7.4)	2.1	(1.2-3.6)
A-B-Cw-DQB1	0201-4402-0501-xxxx-0301	23	(4.6)	41	(8.4)	2.0	(1.2-3.3)
A-B-DRB1-DQB1	0201-4402-xxxx-0401-0301	20	(4.0)	34	(6.9)	1.9	(1.1-3.4)
B-Cw-DRB1-DQB1	xxxx-4402-0501-0401-0301	22	(4.4)	42	(8.6)	2.2	(1.3-3.7)
A-B-Cw	0201-4402-0501-xxxx-xxxx	43	(8.6)	59	(12.0)	1.5	(1.0-2.3)
A-B-DQB1	0201-4402-xxxx-xxxx-0301	27	(5.4)	50	(10.2)	2.1	(1.3-3.4)
A-B-DRB1	0201-4402-xxxx-0401-xxxx	22	(4.4)	38	(7.8)	2.0	(1.2-3.4)
A-Cw-DQB1	0201-xxxx-0501-xxxx-0301	23	(4.6)	44	(9.0)	2.1	(1.3-3.6)
A-Cw-DRB1	0201-xxxx-0501-0401-xxxx	20	(4.0)	37	(7.6)	2.1	(1.2-3.7)
A-DRB1-DQB1	0201-xxxx-xxxx-0401-0301	30	(6.0)	54	(11.0)	2.1	(1.3-3.3)*
B-Cw-DQB1	xxxx-4402-0501-xxxx-0301	30	(6.0)	59	(12.0)	2.2	(1.4-3.6)*
B-Cw-DRB1	4402-0501-0401	25	(5.0)	49	(10.0)	2.3	(1.4-3.8)*
B-DRB1-DQB1	4402-0401-0301	24	(4.8)	45	(9.2)	2.1	(1.3-3.6)
Cw-DRB1-DQB1	0501-0401-0301	22	(4.4)	43	(8.8)	2.2	(1.3-3.8)
A-B	0201-4402	51	(10.2)	71	(14.5)	1.6	(1.1-2.3)
A-DQB1	0201-0301	74	(14.7)	108	(22.0)	1.7	(1.2-2.3)*
B-Cw	4402-0501	58	(11.6)	90	(18.4)	1.8	(1.3-2.6)*
B-DQB1	4402-0301	35	(7.0)	73	(14.9)	2.4	(1.6-3.7)*
B-DRB1	4402-0401	27	(5.4)	52	(10.6)	2.3	(1.4-3.7)*
Cw-DQB1	0501-0301	35	(7.0)	64	(13.1)	2.1	(1.4-3.2)*
Cw-DRB1	0501-0401	25	(5.0)	52	(10.6)	2.4	(1.5-4.0)*
DRB1-DQB1	0401-0301	55	(10.2)	81	(15.1)	1.7	(1.1-2.4)
B-DRB1-DQB1	4402-1101-0301	3	(0.6)	28	(5.7)	10.0	(3.0-33.3)*
B-DRB1	4402-1101	3	(0.6)	29	(5.9)	10.5	(3.2-34.8)*
DRB1-DQB1	1101-0301	30	(5.6)	64	(11.9)	2.3	(1.4-3.6)*
B-Cw-DQB1	0702-0702-0301	24	(4.8)	53	(10.8)	2.5	(1.5-4.2)*

Loci	Alleles	Controls (N=502)		Cases (N=490)		OR ¹	95% CI
		n	%	n	%		
B-DQB1	0702-0301	24	(4.8)	58	(11.8)	2.8	(1.7-4.5)*
Cw-DQB1	0702-0301	28	(5.6)	59	(12.0)	2.3	(1.4-3.7)*
A-DQB1	0301-0501	28	(5.6)	42	(8.6)	1.7	(1.0-2.8)
A-B	0201-1501	43	(8.6)	16	(3.3)	0.4	(0.2-0.7)*
A-Cw	1101-0401	31	(6.2)	16	(3.3)	0.5	(0.3-1.0)
A-DQB1	0201-0201	54	(10.8)	27	(5.5)	0.5	(0.3-0.8)*
B-Cw	0801-0701	122	(24.3)	84	(17.1)	0.7	(0.5-0.9)
Cw-DQB1	0701-02	110	(21.9)	76	(15.5)	0.7	(0.5-1.0)
DRB1-DQB1	0301-02	136	(25.3)	100	(18.6)	0.7	(0.5-1.0)

¹Odds ratios are adjusted for race. **Bolding** represents significant associations, although rounding sometimes caused confidence intervals to include 1.0.

* Holm test p-value < 0.05, result is significant after adjusting for multiple comparisons.

Table 5
Risk of SCC, adenocarcinoma of the cervix, and squamous cell vulvar cancer associated with selected class I and II multilocus genotypes

	Controls (N=502)			SCC Cervix (n=490)			Adenocarcinoma (n=537)			Controls ² N=637			SCC Vulvar (n=424)			
	n	(%)		n	(%)	OR ¹ (95% CI)	n	(%)	OR ¹ (95% CI)	n	(%)		n	(%)	OR ¹ (95% CI)	
B*4402-DRB1*1101-DQB1*0301																
X-X-X	312	(62.0)	ref	253	(52.0)	ref	309	(57.5)	ref	380	(59.7)	ref	224	(52.8)	ref	
4402-X-X	35	(7.0)	1.4 (0.9-2.3)	38	(7.8)	1.4 (0.9-2.3)	43	(8.0)	1.3 (0.8-2.0)	46	(7.2)	0.8 (0.5-1.4)	23	(5.4)	0.8 (0.5-1.4)	
X-1101-X	3	(0.6)	--	0	(0.0)	--	1	(0.2)	0.3 (0.0-2.7)	5	(0.8)	0.4 (0.0-3.1)	1	(0.2)	0.4 (0.0-3.1)	
X-X-0301	92	(18.3)	1.2 (0.9-1.7)	91	(18.7)	1.2 (0.9-1.7)	100	(18.6)	1.1 (0.8-1.5)	112	(17.6)	0.8 (0.6-1.2)	56	(13.2)	0.8 (0.6-1.2)	
4402-1101-X	0	(0.0)	--	1	(0.2)	--	0	(0.0)	--	0	(0.0)	--	0	(0.0)	--	
4402-X-0301	32	(6.4)	1.8 (1.1-3.0)*	45	(9.2)	1.8 (1.1-3.0)*	37	(6.9)	1.2 (0.7-2.0)	47	(7.4)	1.4 (0.9-2.3)	40	(9.4)	1.4 (0.9-2.3)	
X-1101-0301	26	(5.2)	1.5 (0.8-2.5)	31	(6.4)	1.5 (0.8-2.5)	33	(6.1)	1.3 (0.8-2.2)	39	(6.1)	2.5 (1.6-3.9)*	58	(13.7)	2.5 (1.6-3.9)*	
4402-1101-0301	3	(0.6)	11.5 (3.5-38.5)*	28	(5.7)	11.5 (3.5-38.5)*	14	(2.6)	4.9 (1.4-17.2)*	8	(1.3)	4.7 (2.0-10.7)*	22	(5.2)	4.7 (2.0-10.7)*	
B*0702-Cw*0702-DQB1*0301																
X-X-X	237	(47.1)	ref	201	(41.0)	ref	225	(41.9)	ref	296	(46.5)	ref	161	(38.0)	ref	
0702-X-X	2	(0.4)	--	0	(0.0)	--	2	(0.4)	1.1 (0.2-7.9)	3	(0.5)	1.8 (0.4-9.2)	3	(0.7)	1.8 (0.4-9.2)	
X-0702-X	10	(2.0)	0.9 (0.4-2.3)	11	(2.2)	0.9 (0.4-2.3)	11	(2.0)	1.1 (0.4-2.6)	15	(2.4)	0.5 (0.2-1.5)	4	(0.9)	0.5 (0.2-1.5)	
X-X-0301	125	(24.9)	1.3 (0.9-1.7)	132	(26.9)	1.3 (0.9-1.7)	140	(26.1)	1.2 (0.9-1.6)	167	(26.2)	1.4 (1.0-1.9)	126	(29.7)	1.4 (1.0-1.9)	
0702-0702-X	101	(20.1)	1.0 (0.7-1.4)	82	(16.7)	1.0 (0.7-1.4)	115	(21.4)	1.2 (0.9-1.7)	117	(18.4)	1.3 (0.9-1.8)	80	(18.9)	1.3 (0.9-1.8)	
0702-X-0301	0	(0.0)	--	5	(1.0)	--	1	(0.2)	--	1	(0.2)	--	0	(0.0)	--	
X-0702-0301	4	(0.8)	1.2 (0.3-4.4)	6	(1.2)	1.2 (0.3-4.4)	6	(1.1)	1.3 (0.3-4.7)	5	(0.8)	--	0	(0.0)	--	
0702-0702-0301	24	(4.8)	2.7 (1.6-4.6)*	53	(10.8)	2.7 (1.6-4.6)*	37	(6.9)	1.7 (1.0-2.9)	33	(5.2)	2.8 (1.7-4.5)*	50	(11.8)	2.8 (1.7-4.5)*	
B*0801-DRB1*0301-DQB1*0201																
X-X-X	346	(69.9)	ref	379	(77.8)	ref	396	(74.0)	ref	450	(71.2)	ref	321	(76.4)	ref	
0801-X-X	25	(5.1)	0.6 (0.3-1.1)	15	(3.1)	0.6 (0.3-1.1)	21	(3.9)	0.7 (0.4-1.4)	30	(4.7)	1.1 (0.6-1.9)	23	(5.5)	1.1 (0.6-1.9)	
X-0301-X	0	(0.0)	--	0	(0.0)	--	0	(0.0)	--	0	(0.0)	--	0	(0.0)	--	
X-X-0201	0	(0.0)	--	0	(0.0)	--	0	(0.0)	--	0	(0.0)	--	0	(0.0)	--	
0801-0301-X	1	(0.2)	0.7 (0.0-11.3)	1	(0.2)	0.7 (0.0-11.3)	0	(0.0)	--	1	(0.2)	1.4 (0.1-22.2)	1	(0.2)	1.4 (0.1-22.2)	
0801-X-0201	0	(0.0)	--	0	(0.0)	--	0	(0.0)	--	0	(0.0)	--	0	(0.0)	--	
X-0301-0201	29	(5.9)	0.5 (0.3-1.0)	16	(3.3)	0.5 (0.3-1.0)	26	(4.9)	0.8 (0.5-1.4)	36	(5.7)	0.9 (0.5-1.5)	23	(5.5)	0.9 (0.5-1.5)	
0801-0301-0201	97	(19.6)	0.7 (0.5-1.0)	73	(15.0)	0.7 (0.5-1.0)	92	(17.2)	0.8 (0.6-1.2)	115	(18.2)	0.6 (0.4-0.9)	52	(12.4)	0.6 (0.4-0.9)	
DRB1*13-DQB1*06																

	Controls (N=502)			SCC Cervix (n=490)			Adenocarcinoma (n=537)			Controls ² (N=637)			SCC Vulvar (n=424)		
	n	(%)	OR ¹ (95% CI)	n	(%)	OR ¹ (95% CI)	n	(%)	OR ¹ (95% CI)	n	(%)	OR ¹ (95% CI)	n	(%)	OR ¹ (95% CI)
X-X	60	(10.1)	ref	61	(16.1)	ref	58	12.7	ref	70	(11.7)	ref	54	(14.2)	ref
DRB1*13-X	2	(0.3)	(0.0)	0	(0.0)	0	0	0	0	3	(0.5)	(0.0)	0	(0.0)	(0.0)
X-DQB1*06	77	(12.9)	(18.7)	71	(18.7)	0.9 (0.6-1.4)	77	16.8	1.1 (0.7-1.7)	84	(14.1)	1.1 (0.7-1.8)	73	(19.3)	1.1 (0.7-1.8)
X-DQB1*0301	71	(11.9)	(26.6)	101	(26.6)	1.4 (0.9-2.2)	87	19	1.2 (0.8-2.0)	91	(15.2)	1.0 (0.6-1.5)	68	(17.9)	1.0 (0.6-1.5)
X-DQB1*0302	56	(9.4)	(13.2)	50	(13.2)	0.9 (0.5-1.5)	52	11.4	1.0 (0.6-1.7)	65	(10.9)	0.9 (0.5-1.5)	46	(12.1)	0.9 (0.5-1.5)
X-DQB1*0501	44	(7.4)	(10.0)	38	(10.0)	0.9 (0.5-1.5)	47	10.3	1.1 (0.6-1.9)	54	(9.0)	0.3 (3.7)	14	(3.7)	0.3 (0.2-0.7)*
DRB1*13-DQB1*06	61	(10.2)	(9.0)	34	(9.0)	0.6 (0.3-1.0)	35	7.6	0.6 (0.3-1.0)	77	(12.9)	0.4 (0.2-0.7)*	25	(6.6)	0.4 (0.2-0.7)*
DRB1*13-DQB1*0301	7	(1.2)	(2.4)	9	(2.4)	1.4 (0.5-3.9)	2	0.4	0.3 (0.1-1.6)	7	(1.2)	1.3 (1.8)	7	(1.8)	1.3 (0.4-3.9)
DRB1*13-DQB1*0302	1	(0.2)	(0.3)	1	(0.3)	0.7 (0.0-12.2)	0	0	0	1	(0.2)	(0.0)	0	(0.0)	(0.0)
DRB1*13-DQB1*0501	0	(0.0)	(0.0)	0	(0.0)	0	1	0.2	0	0	(0.0)	(0.0)	0	(0.0)	(0.0)
DRB1*13-DQB1*06/*0301	52	(8.7)	(14.8)	56	(14.8)	1.1 (0.6-1.8)	37	8.1	0.8 (0.4-1.3)	70	(11.7)	1.1 (15.3)	58	(15.3)	1.1 (0.6-1.7)
DRB1*13-DQB1*06/*0302	30	(5.0)	(7.7)	29	(7.7)	1.0 (0.5-1.8)	37	8.1	1.3 (0.7-2.4)	42	(7.0)	0.7 (6.1)	23	(6.1)	0.7 (0.4-1.3)
DRB1*13-DQB1*06/*0501	23	(3.9)	(6.3)	24	(6.3)	1.0 (0.5-2.1)	25	5.5	1.2 (0.6-2.3)	33	(5.5)	0.4 (0.2-0.9)	11	(2.9)	0.4 (0.2-0.9)

¹ Odds ratios are adjusted for race. Bolding represents significant associations, although rounding sometimes caused confidence intervals to include 1.0.

² Controls for the vulvar cancer study include women with a history of hysterectomy.

* Holm test p-value < 0.05, result is significant after adjusting for multiple comparisons.