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Nucleotide variation in IL10 and IL12 and their receptors and cervical and vulvar cancer risk: a hybrid case-parent triad and case-control study

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Abstract

Given the important role of cell mediated immunity in viral clearance and control of premalignant lesions, we hypothesize that variation in the IL12/IL10 cytokine and cytokine receptor genes may influence cervical and vulvar cancer risk. We evaluated 76 tagSNPs from 7 candidate genes (*IL10, IL12A, IL12B, IL10RA, IL10RB, IL12RB1, and IL12RB2*) in case-parent sets (n=43 cervical squamous cell carcinoma (SCC), n=96 cervical adenocarcinoma, n=53 vulvar SCC), additional cases (n=356 cervical SCC, n=406 cervical adenocarcinoma, and n=473 vulvar SCC) and population based controls (1,111). We calculated log-additive odds ratios (ORs) and 95% confidence intervals (CIs) for the association between tagSNP and cancer risk using a pseudo-likelihood based method which combined genotype information on cases, parents, and population controls. After correction for multiple comparisons, we identified several statistically significant SNP associations. Cervical SCC risk was associated with the minor alleles of the *IL10RA* rs9610 3′ UTR SNP (OR=1.76, 95% CI= 1.15–2.68) and two synonymous *IL12RB2* SNPs (rs4297265, OR=0.46, 95% CI=0.26–0.82; rs2229546, OR=0.43, 95% CI=0.21–0.87). Cervical adenocarcinoma risk was associated with the minor alleles of the *IL10RA* rs4252314 intronic SNP (OR=2.23, 95% CI = 1.26–3.96) and *IL12RB1* rs11575934 non-synonymous SNP (OR=1.51, 95% CI=1.12–2.05). Finally, the minor allele of the *IL12B* rs3181224 3′ UTR SNP was associated with a reduced risk of vulvar SCC (OR=0.30, 95% CI=0.12–0.74). These results raise the possibility

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Novelty Statement: This is the first manuscript reporting on cervical and vulvar cancer risk in relation to genetic variation in IL12 and IL10 and their receptors; two immunoregulatory cytokines with opposite effects on the Th1/Th2 cytokine balance. We employed a non-traditional study design which utilizes genetic data from cases, their biological parents, and population controls.

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that a shift in the balance of the immune response due to genetic variants in key cytokine genes could influence the development of cervical and vulvar cancer.

Keywords

Cervical cancer; vulvar cancer; case-parent; interleukin 12; interleukin 10

Introduction

Over the last decade, there has been substantial progress in our understanding of the etiology and prevention of anogenital cancers. Despite this fact, these cancers are still responsible for considerable mortality and morbidity worldwide.[1] Furthermore, since the 1970's, the incidence of invasive cervical adenocarcinoma and vulvar squamous cell carcinoma has been on the rise in the United States.[2, 3] Persistent oncogenic human papillomavirus (HPV) infection is a necessary cause of all cervical cancers and a large subset of vulvar cancers.[4] Those vulvar cancers that are etiologically linked to HPV primarily affect younger women and are associated with nonkeratinizing basaloid or warty vulvar intraepithelialneoplasia. These neoplasias are very similar to cervical squamous intraepithelialneoplasia, and are associated with similar HPV types and co-factors.[5, 6]

The immune response is important in cervical and vulvar cancer pathogenesis. Although neutralizing antibodies have a pivotal role in the prevention of initial HPV infection, cell-mediated immune response is important for viral clearance and control of premalignant lesions. The homeostatic balancing of key T helper (Th) cytokines controls lymphocyte development and differentiation, influencing the type of cell mediated immune response. Th1 cytokines, such as IL12, enhance T-cell mediated immunity, while IL10 (a Th2 cytokine) down-regulates the expression of IL12 and other Th1cytokines. There is a large body of literature showing a shift in circulating and cervical cytokine profiles from Th1 to Th2, specifically decreased IL12 and increased IL10, with HPV infection/persistence and increasing grade of cervical disease.[7–11] A growing body of literature also highlights the important role of immunogenetics in HPV-related carcinogenesis.[12–15]

Studies show that IL10 and IL12 cytokine expression is partly genetically determined.[16–19] However, whether *IL10* and *IL12* SNPs are associated with the risk of HPV associated anogenital cancers has not been adequately addressed. Thus, we investigated the association of common variation in these genes and their receptors with cervical and vulvar cancer risk in a population-based, hybrid case-control and case-parent study.

Materials and Methods

Study design

We designed a candidate gene association study combining case-parent triad and traditional case-control approaches. Given the genotypes of biological parents, the distribution of genotypes for a polymorphism among probands should conform to Mendelian expectation if there is no association between the polymorphisms and disease risk. Evidence for a genetic association is inferred from a non-Mendelian distortion in the probands' genotype frequencies. The precision of this association is enhanced by including cases without parents (non-proband cases) and unrelated controls.[20] Homogeneity of the ORs in the case-parent controls and case-unrelated controls is an assumption of this method.

Study population

This study was conducted within a large population-based case-control study of host and environmental factors related to anogenital cancer risk.[5, 21] Briefly, the case-control study included 18 to 74 year-old Seattle metropolitan residents, with incident invasive squamous cell carcinoma (SCC) of the cervix (ICD-O 8010–8081), invasive or *in situ* adenocarcinoma (AC) of the cervix (ICD-O 8140–8480) and invasive or *in situ* SCC of the vulva (ICD-O 8010, 8070–8077, 8081) diagnosed between January 1986 and June 1998, or between January 2000 and December 2004. Cases were ascertained through the Cancer Surveillance System, a population-based registry that is a part of the National Cancer Institute's Surveillance, Epidemiology, and End Results program.[22] Controls were identified and selected using a one-step modification of the Waksberg-Mitofsky method of random-digit telephone dialing and frequency matched to cases on age and county of residence.[23]

Probands are a subset of the cases described in the previous paragraph. Eligible probands were cases diagnosed at ages 18 to 49 between January 2001 and December 2004 for cervical cancer, and January 2001 and December 2003 for vulvar cancer. Ages and diagnosis dates were restricted in an attempt to improve the likelihood that parents were alive and thus available for participation when parent recruitment began in May, 2003. A biological parent of a proband was eligible for this study if his or her daughter provided consent to contact him/her and parental contact information. Parents were not eligible if they resided outside of the United States or did not speak English.

Data and specimen collection

Standardized interviews were administered to collect information on demographic and other characteristics with a known or suspected relationship to anogenital cancer. Venous blood samples were collected into EDTA-containing tubes to provide buffy coats from which DNA could be extracted for genetic polymorphism assays, and stored at −80°C. A small proportion of study participants (3%) preferred to donate a buccal rinse sample in place of blood.

Response proportions

Among the 1,189 eligible cervical SCC patients identified for the case-control study, 744 (62.6%) were interviewed and among those interviewed 674 (90.6%) provided a specimen from which DNA could be obtained. For cervical adenocarcinoma (*in situ* and invasive), among the 805 eligible patients, 553 (68.7%) were interviewed and 520 (94.0%) provided a specimen. A similar proportion, 807 of the 1194 eligible vulvar SCC patients (67.6%) was interviewed, and DNA was collected from 73.4% of participating patients. Among the eligible population-based control women who were approached, 67 % agreed to participate in the interview, and 83.9% (N=1,372) of those interviewed donated a blood sample from which DNA could be obtained. Sixty-five % of eligible probands who provided a specimen provided consent to contact at least one biological parent. Among the 337 parents for whom we had complete contact information and who resided within the U.S., 1 (0.3%) died prior to contact, 31 (9.2%) refused to participate, 6 (1.8%) consented to participate but did not submit a specimen, and 299 (88.7%) consented to participate and provided a blood or buccal specimen. DNA was successfully isolated from samples belonging to 295 of these 299 parents.

Women whose self-reported race was anything other than White were excluded from the current study because they comprised less than 10% of the study population, precluding meaningful sub-group analyses stratified by race while increasing the possibility of bias due to population stratification if combined in an analysis without genomic adjustment. All tables present data and results from analysis of the White study population only.

Candidate gene tagSNP selection & genotyping

TagSNPs were selected within each candidate gene including all exons, introns, and approximately 5 kb of 3′ and 5′ flanking sequence with an attempt to cover all known common nucleotide variation within those regions. We included genes coding for all subunits of IL10 (*IL10*), IL12 (*IL12A, IL12B)*, the IL10 receptor (*IL10RA, IL10RB*), and the IL12 receptor (*IL12RB1, IL12RB2).* Information on nucleotide variation for these genes was obtained from the European descent population in the SeattleSNPs Variation Discovery Resource and the International HapMap Project.[24, 25] To select tagSNPs, a pairwise $r^2 \geq 0.80$ was used to delineate groups of highly correlated SNPs with a minor allele frequency of 5% or greater, primarily using Tagger and Snagger.[26, 27] Generally one SNP per group of correlated SNPs was selected for genotyping, however, at times redundant SNPs were selected to ensure adequate gene coverage in case of SNP genotyping failure. Some additional SNPS were also selected with MAF < 5% if they were located in an exon or of interest based on previous literature.

Genomic DNA was extracted from buffy coat aliquots from blood samples, or cell pellets from buccal samples, using a modified phenol-chloroform method. SNP genotyping was performed primarily using the Illumina GoldenGate® custom assay. A smaller proportion of SNPs were genotyped using TaqMan® Assays (Applied Biosystems Incorporated) or KASPar SNP Genotyping Assays (an allele-specific PCR system, Kbiosciences). Several SNPs were genotyped using more than one of these assays for quality control purposes. Call rates for passing SNPs were very high for all three assays: GoldenGate assay (mean=0.998, range=0.977 to 1.00), TaqMan (mean=0.976, range=0.958 to 0.987), and KASPar (mean=0.989, range=0.984 to 0.994), and the concordance between assays was very high (0.987–0.999). The final set of tagSNPs included in this study was selected based on successful genotyping on ≥90% of the samples and genotype concordance within pairs of duplicate samples ≥90%. After excluding SNPs failing our quality control requirements and monomorphic SNPs, genotype data were available on 100 tagSNPs in our candidate genes: 73 genotyped using the GoldenGate assay, 15 genotyped using the TaqMan assays, and 12 genotyped using the KASPar assay. We further screened our genotyped SNPs to identify pairs or groups of SNPs that were in high linkage disequilibrium (LD, $r^2 \geq 0.80$) in our study population. We selected only one SNP per group of SNPs in high LD to present in our tables, for a total of 76 tagSNPs.

Specimens from study participants, including 7% replicate DNA aliquots, were distributed randomly throughout the reaction plates. Laboratory technicians were blinded to all research information about the samples, including the identities of the quality control replicate aliquots. Analysis of our replicate samples revealed a low discordance proportion ranging from 0 to 0.4%. A total of 8 cervical cancer cases, 1 vulvar cancer case, and 8 population controls were dropped due to genotyping failure for the majority of SNP.

HPV typing

Tissue blocks from 265 out of 399 (66.4%) cervical SCC cases, 346 out of 502 (68.9%) cervical adenocarcinoma cases, and 400 out of 526 (76.0%) vulvar SCC cases were obtained for HPV typing. PCR-RFLP methods were used for typing, with MY09/MY11 L1 consensus and HPV16 and HPV18 E6 type-specific primers, and coamplification of 236 or 536 bp fragments of β-globin to test for sample integrity.[28]

Data analysis

Case-parent sets found to violate Mendelian transmission for more than two SNPs were excluded from all analyses. TagSNP genotypes were tested for consistency with Hardy-

Weinberg equilibrium (HWE) within the non-parental control sample using a Pearson's χ^2 *p* value.

To obtain odds ratio (ORs) and 95% confidence intervals (CIs) for the association of each tagSNP with cervical and vulvar cancer risk on a log-additive scale, we used a pseudo-likelihood based method that utilized genotype information on probands, parents, non-proband cases, and population controls implemented in the software package Hybrid.[29] The cervical cancer control group was restricted to women without prior hysterectomy, thus reflecting the population from which the cases arose. In families where only one parent was available (dyads), missing parental genotypes were imputed by Hybrid through a combination of the EM algorithm and multiple imputation methods. To prioritize our results for interpretation and future studies, we chose to implement the Holm procedure, a sequentially rejective adaptation to the Bonferroni method, to correct for multiple testing.[30] Holm-adjusted p-values are included in Table 3 corresponding to associations where uncorrected p-values are less than or equal to 0.05. Associations with uncorrected p-values less than or equal to 0.05 were considered nominally significant, while associations with Holm-adjusted p-values less than 0.05 were considered statistically significant. ORs for tagSNPs with nominally or statistically significant associations utilizing all of the genotype information described in the previous paragraph were also calculated separately for case-parent and case-unrelated controls in Hybrid (Supplementary Table).

TagSNPs with nominally or statistically significant associations in either the cervical SCC or cervical adenocarcinoma models were also included in a post hoc analysis with HPV16 and HPV18 positive cervical cancer as an outcome. Cases positive for both HPV16 and HPV18 were included in both analyses. Similarly, tagSNPs with nominally or statistically significant associations in the vulvar SCC models were included in HPV positive only vulvar SCC analysis. Holm's correction was not applied to these post hoc analyses.

Results

Two case-parent sets were dropped due to non-Mendelian transmission. Cervical SCC analyses included 43 invasive cervical SCC proband cases with 59 parents (16 triads/27 dyads), 356 non-proband cases, and 861 population-based controls. Cervical AC analyses included 79 *in situ* proband cases and 17 invasive proband cases with 151 parents (55 triads/41 dyads), 241 *in situ* non-proband cases and 165 invasive non-proband cases, and 861 population-based controls. Vulvar SCC analyses included 49 *in situ* proband cases and 4 invasive proband cases with 70 parents (17 triads/36 dyads), 402 *in situ* non-proband cases and 71 invasive non-proband cases, and 1,111 population-based controls. There were higher proportions of *in situ* cervical AC and vulvar SCC proband cases compared with non-proband cases because probands were intentionally selected to be young, and *in situ* carcinomas tend to be diagnosed at a younger age (Table 1).

Vulvar SCC cases were more than twice as likely as controls to be current cigarette smokers, while cervical SCC cases were slightly more likely, and cervical AC cases were slightly less likely, to be current smokers compared to controls (Table 1). All cases tended to have more lifetime sexual partners and a history of oral contraceptive use compared to controls. Among the 76 SNPs included in this study, 21(27.6%) were located in UTRs or exons, 18 (23.7%) were in flanking regions, and 37 (48.7%) were intronic (Table 2). The MAF in controls ranged from 0.01 to 0.50 (median=0.24). One SNP (*IL12RB2* rs3762315) had a HWE *p* value ≤0.001.

None of the *IL10* SNPs were significantly associated with cervical SCC or AC, but one flanking 5′ UTR SNP was associated with vulvar SCC (rs1800871, OR=1.62, 95%

CI=1.04–2.54, Table 3). In *IL10RA*, one intronic SNP was associated with cervical SCC (rs4252253, OR=0.38, 95% CI=0.16–0.91), a second intronic SNP was statistically significantly associated with cervical AC (rs4252314, OR=2.23, 95% CI = 1.26–3.96), and a 3′ UTR SNP was statistically significantly associated with increased risk of cervical SCC (rs9610, OR=1.76, 95% CI= 1.15–2.68). None of the *IL10RB* SNPs were significantly associated with cervical or vulvar cancer, with the exception of one intronic SNP whose minor allele was associated with a 50% reduced risk of cervical SCC (rs2843944, OR=0.50, 95% CI= 0.28–0.91).

Several SNPs were associated with cervical and vulvar cancer in the genes coding for IL12 and IL12 receptor. Among the most notable associations were a non-synonymous SNP in *IL12B* (rs3213119) whose minor allele was associated with a non-significant 44–55% reduced risk of cervical SCC, cervical AC, and vulvar SCC. Another *IL12B* intronic SNP (rs2569253, minor allele) was associated with a non-significant reduced risk of both cervical SCC (OR=0.64, 95% CI=0.39–1.04) and vulvar SCC (OR=0.69. 95% CI=0.45–1.07). The minor allele of a flanking 3′UTR SNP was associated with an increased risk of cervical SCC (rs3181225, OR=1.85, 95% CI=1.07–3.21), while the minor allele of another flanking 3′UTR SNP was associated with a statistically significant reduced risk of vulvar SCC (rs3181224, OR=0.30, 95% CI=0.12–0.74). Cervical SCC was also inversely associated with the minor allele of another intronic SNP (rs730691, OR=0.57, 95% CI=0.32–1.01).

In *IL12RB1*, the minor allele of a flanking 3′UTR SNP was associated with an increased risk of cervical SCC (rs17885963, OR=2.0, 95% CI=1.03–3.91), while the minor allele of another 3′UTR SNP was associated with a reduced risk of vulvar SCC (rs3746190, OR=0.70, 95% CI=0.49–1.01). Additionally, cervical AC risk was inversely associated with the minor allele of an intronic SNP (rs383483, OR=0.75, 95% CI=0.55–1.01) and positively statistically significantly associated with the minor allele of a non-synonymous SNP (rs11575934, OR=1.51, 95% CI=1.12–2.05). Cervical SCC risk was inversely statistically significantly associated with the minor allele of two synonymous SNPs (rs4297265, OR=0.46, 95% CI=0.26–0.82 and rs2229546, OR=0.43, 95% CI=0.21–0.87).

In general, the HPV16 positive stratified cervical cancer SNP associations were similar to results from the cervical SCC models given the high proportion of HPV16 positive versus HPV18 positive cervical SCC (49% versus 10%, Table 4). An exception was a strong inverse association seen between the minor alleles of each of the two synonymous SNPs in *IL12RB2* (rs4297265 and rs2229546) and cervical SCC in contrast to HPV18 positive cervical cancers being positively associated with the minor alleles of these SNPs. Also, the strong positive association that was observed between *IL10RA* SNP rs4252314 minor allele and cervical AC, was seen in both HPV16 positive and HPV18 positive cervical cancers. The HPV positive vulvar SCC SNP associations for rs1800871, rs3181224, and rs3746190 were in the same direction and of similar magnitude to results for all vulvar SCC (data not shown).

Discussion

We report the first comprehensive study of cervical and vulvar cancer risk in relation to variation in genes related to IL12 and IL10, two immunoregulatory cytokines with opposite effects on the Th1/Th2 balance. The underlying hypothesis of this study is that a cell mediated immune response associated with increased expression of Th1 cytokines, such as IL12, combats pathological events in the cervix and vulva that may be associated with HPV persistence and pathogenesis of cancer; IL10 inhibits this response, and the ability to mount a Th1 response is partly genetically determined.

IL12 is an important modulator of the immune response and provides the stimulus for CD4+ T cells and NK cells to differentiate toward a cell mediated immunity Th1 phenotype. IL12 is produced primarily by antigen-presenting cells and its functions are mediated through high-affinity binding to the IL12 receptor expressed on activated T and NK cells. The biologically active form of IL12 is a heterodimer (p70) composed of two unrelated disulfide-linked subunits, p35 and p40, which are encoded by *IL12A* and *IL12B,* respectively.[31]

Among the most notable findings from this study was the observation that *IL12B* SNPs, particularly SNPs in the 3′ region (rs3181225, rs3181224, rs3212227) and a non-synonymous SNP (rs3213119) were associated with risk of cervical and vulvar cancers. These results are particularly interesting in the context of literature describing functional and disease associations of *IL12B* SNPs. The rs3212227 minor allele is associated with increased secretion of IL12 p70 by stimulated human monocytes.[17] Thus, the 50% reduced risk of cervical SCC is consistent with our hypothesis that variation in this pro-Th1 cytokine gene bolsters the functional immune response against HPV infection and related carcinogenesis. The rs3212227 minor allele has also been associated with a decreased risk of a number of different infectious and Th1/Th17-mediated autoimmune diseases.[32–34]

A few studies to date have examined the association *IL12B* SNPs and cervical cancer. Women who were heterozygous for the rs3212227 minor allele were at increased cervical cancer risk in one study,[35] but not in other studies.[36, 37] Carriers of the rs2569254 minor allele (not genotyped in our study) were at significantly increased risk for cervical cancer.[37] Rs2569254 is an intronic *IL12B* SNP which is in strong LD with rs3181225, a 3′flanking SNP whose minor allele was significantly increased risk of cervical SCC in our study. The minor allele of another 3′flanking SNP, rs3181224, was associated with reduced risk of vulvar SCC in our study. Also, the minor allele of intronic SNP rs730691, which is in LD with 3′flanking SNP rs2116821in HapMap (not genotyped in our study), was associated with reduced risk of cervical SCC in our study. Taken together, these findings suggest that *IL12B* SNPs, particularly those in the 3′ region, are important in cervical and vulvar carcinogenesis.

Although there are some differences in the implicated SNP, it is notable that several studies, including ours, have found an association between 3′ region SNPs in *IL12B* and diseases related to infections or autoimmunity, including cervical and vulvar cancer, given the importance of this region in post-transcriptional regulation. Furthermore, chromosome 5q31, where *IL12B* is located, contains numerous immunoregulatory genes, and the true disease associated variant could be in one of those genes and in LD with SNPs in the 3′ region of *IL12B*. For example, *EBF1,* a principal regulator of B cell development, is about 200kb downstream of *IL12B* and according to HapMap CEU data there is LD between several pairs of SNPs in *IL12B* and *EBF1* (r^2 ranging from 0.52–0.73). Importantly, genetic variation in *EBF1* has been associated with several infections and immune related disorders.[38]

The minor allele of *IL12B* rs3213119 was associated with a 44–50% reduced risk of cervical and vulvar cancers in our study, although these associations did not reach statistical significance. This SNP results in valine to phenylalanine substitution, which is predicted to affect protein function and designated “possibly damaging” by Polyphen and “damaging” by SIFT.[39, 40] Further studies are needed to elucidate the functional consequences of this SNP, including effects on protein folding, formation of IL12 homodimers/heterodimers, and binding to the IL12 receptor.

We found several SNPs in the IL12 receptor genes to be significantly associated with cervical cancer risk. One of the most noteworthy findings was that the minor allele of a non-

synonymous SNP in *IL12RB1* (rs11575934, glutamine to arginine) was associated with a significant increased cervical AC risk. In previous studies, the minor allele of rs11575934 was associated with a more than 2-fold increased risk of tuberculosis and functional data show that IFNγ (another pro-Th1 immunity cytokine) production, T lymphocyte proliferation, and NK lytic activity in response to IL12 were significantly lower in minor allele homozygotes compared to major allele homozygotes.[41] Importantly, this SNP is in near complete LD (r^2=0.97–0.99) with several other interesting SNPs in *IL12RB1* including a non-synonymous SNP (rs375947) and synonymous SNP (rs17852635). However, neither non-synonymous SNPs (rs11575934 and rs375947) are predicted to have functional effects.[39, 40] In *IL12RB2*, the minor alleles of several SNPs were associated with reduced risk of cervical SCC, including two synonymous SNPs, rs4297265 and rs2229546. Interestingly, the minor allele of rs4297265 was associated with a similar reduced risk of HPV16 positive cervical cancer, while rs2229546 was associated with an increased risk of HPV18 positive cervical cancer. Further studies are needed to confirm these associations and elucidate the potential role of these SNPs on IL12 receptor function.

There were also a few notable associations between cervical and vulvar cancer risk and SNPs in *IL10* and its receptor in our study. IL10 is a Th2 cytokine with suppressive effects on cell-mediated immunity, including the inhibition of Th1 cytokines such as IL12. In addition to its immunsuppressive effects, IL10 can induce transcription of the HPV16 E7 oncoprotein.[42] Fifty percent of the variability of *IL10* expression is thought to be due to genetic variance, and functional studies indicate that certain haplotypes made up of several *IL10* 5′ region SNPs are associated with IL10 levels in circulation.[18, 19] SNPs in *IL10*, *IL10RA*, or *IL10RB* may also influence IL10 levels in the cervix.[43]

Rs1800871, the minor allele of which was associated with an increased risk of vulvar SCC in our study, is a 5′ region SNP. Rs1800871, and other SNPs that are in high LD with this SNP (including rs1800872 and rs100893) have been associated with HPV clearance/persistence and risk of cervical cancer and precursor lesions in some studies,[44–46] but not others.[47, 48] To our knowledge, no previous studies have examined the association between genetic variation in *IL10RA* and *IL10RB* and cervical or vulvar cancer risk. Interestingly, one study found that several polymorphisms in *IL10RA* were associated with cervical IL10 levels.[43] These SNPs did not include, nor were they in high LD with, the intronic (rs4252314) and 3′ UTR (rs9610) SNPs that we found to be positively associated with cervical cancer.

In our study, several SNPs were associated with cervical SCC, cervical AC, or vulvar SCC, but rarely were similar associations observed across all three sites. Although these three cancers have a common HPV etiology, they exhibit differences in strength, and in some cases direction, of association with major risk factors including specific HPV types and cigarette smoking.[21, 49–51] The vast majority of papers examining the immune response to HPV have focused on cervical cancer, and there is some indication that the immune environment in the squamous epithelium (where cervical SCCs develop) differs from that in the glandular cells (where cervical ACs develop).[52] Additionally, HPV16 positive high grade vulvar lesions and cervical cancer appear to have unique associations certain class I and II human leukocyte antigen alleles,[15, 53] loci that play an important role in regulating T lymphocyte responses to viral proteins. Thus, there may be immunological differences that explain the associations observed in our study across tumor types.

A major assumption of the hybrid design is homogeneity of the ORs in case-parent and case-unrelated controls. For all associations found to be nominally or statistically significant, this assumption appears to be valid (Supplementary Table). Strengths of this study include the population-based recruitment of cases and controls and attempted coverage of all common

genetic variation in our candidate genes. Additionally, the use of a hybrid case-parent triad/case-control study design represents a relatively innovative feature of this study, making use of all available samples thus maximizing statistical power. It is possible that one or more of our SNP associations is modified by risk factors, such as cigarette smoking. However, due to the small number of study subjects and large number of additional hypothesis tests, we opted not to explore effect modification by risk factors to limit the possibility of reporting false positive associations.

In summary, our results raise the possibility that a shift in the balance of the immune response due to genetic variants in key cytokine genes could have consequences for the pathogenesis of cervical and vulvar cancer. As uptake of the prophylactic HPV vaccines continues to rise, the burden of HPV infection and associated neoplasms will be reduced. However, there is a large number of women who will not benefit from the vaccine as they have already acquired HPV infection, are beyond the target age of vaccination, or live in low-resource countries that are challenged by the high cost and distribution of the vaccine. Identification of immunogenetic risk factors that contribute to cervical and vulvar cancer risk may help shed light on the biological mechanisms leading to, and potentially identify women who are at increased risk of developing, these malignancies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations Used

HPV	human papillomavirus
Th	T helper
SCC	squamous cell carcinoma
AC	adenocarcinoma
OR	odds ratio
CI	confidence intervals
LD	linkage disequilibrium

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Table 1

Selected characteristics of the proband cervical and vulvar cancer cases, non-proband cervical and vulvar cancer cases, and population controls, Seattle-Puget Sound Region, 1986–2004

	Cervical squamous cell carcinoma		Cervical adenocarcinoma		Vulvar squamous cell carcinoma		Population controls	
	Proband cases (n=43)	Non-proband cases (n=356)	Proband cases (n=96)	Non-proband cases (n=406)	Proband cases (n=53)	Non-proband cases (n=473)	Cervical cancer[1] (n=861)	Vulvar cancer (n=1,111)
Characteristic								
Tumor behavior, N (%)								
In situ	-	-	79 (82.3)	241 (59.4)	49 (92.5)	402 (85.0)	-	-
Invasive	43 (100)	356 (100)	17 (17.7)	165 (40.6)	4 (7.5)	71 (15.0)	-	-
HPV DNA, N (%)								
Not tested	29 (67.4)	105 (29.5)	54 (56.2)	102 (25.1)	20 (37.7)	106 (22.4)	-	-
Negative	3 (7.0)	19 (5.3)	3 (3.1)	42 (10.3)	3 (5.7)	39 (8.3)	-	-
HPV 16+/18−	11 (25.6)	164 (46.1)	24 (25.0)	118 (29.0)	23 (43.4)	271 (57.3)	-	-
HPV 16−/18+	0	21 (5.9)	10 (10.4)	101 (24.9)	2 (3.8)	20 (4.2)		
HPV 16+/18+	0	21 (5.9)	5 (5.2)	28 (6.9)	1 (1.9)	10 (2.1)		
Other type (s)	0	26 (7.3)	0.0	15(3.7)	4 (7.6)	27 (5.7)	-	-
Mean age (years)	38.0	43.1	32.8	39.7	39.3	48.3	42.6	45.8
Cigarette smoking, N (%)								
Never	24 (55.8)	126 (35.4)	56 (58.3)	210 (51.7)	15 (28.3)	92 (19.5)	468 (54.4)	577 (51.9)
Former	9 (20.9)	100 (28.1)	21 (21.9)	109 (26.9)	6 (11.32)	111 (23.4)	223 (25.9)	(303) 27.3
Current	10 (23.3)	130 (36.5)	19 (19.8)	87 (21.4)	32 (60.38)	270 (57.1)	170 (19.7)	231 (20.8)
Number of lifetime sexual partners, N (%)								
1	3 (7.0)	34 (9.6)	6 (6.3)	52 (12.8)	1 (1.9)	42 (8.9)	204 (23.7)	295 (26.5)
2 to 4	13 (30.2)	109 (30.6)	18 (18.8)	116 (28.6)	6 (11.3)	105 (22.2)	258 (30.0)	344 (30.9)
5 to 14	22 (51.2)	150 (42.1)	46 (47.9)	171 (42.1)	29 (54.7)	204 (43.1)	291 (33.8)	344 (30.9)
≥ 15	5 (11.6)	63 (17.7)	26 (27.1)	67 (16.5)	17 (32.1)	121 (25.6)	108 (12.5)	126 (11.3)
Number of births, N (%)								
0	11 (25.6)	66 (18.5)	43 (44.8)	126 (31.3)	19 (35.9)	132 (27.9)	224 (26.0)	248 (22.3)
1	8 (18.6)	58 (16.25)	14 (14.6)	72 (17.9)	11 (20.8)	90 (19.0)	135 (15.7)	154 (13.9)
2	10 (23.3)	108 (30.25)	30 (31.3)	121 (30.0)	18 (34.0)	117 (24.7)	255 (29.6)	338 (30.4)
≥ 3	14 (32.5)	124 (34.8)	9 (9.4)	84 (20.8)	5 (9.4)	134 (28.3)	247 (28.7)	371 (33.4)

[1]Excluding women with prior hysterectomy

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Table 2

Characteristics of tagSNPs in IL10, IL12A, IL12B, IL10RA, IL10RB, IL12RB1, and IL12RB2

HUGO gene name (region)	dbSNP reference sequence number	SNP context (amino acid change)[1]	Alleles (major/minor)	Minor allele frequency in controls	HWE p-value[2]	Genotyping method[3]	SNPs in high LD[4]
IL10 (1q31)	rs3024505	flanking 3′UTR	G/A	0.15	0.636	OPA	rs3024493
	rs3024498	3′UTR	A/G	0.27	0.037	OPA	
	rs3024509	intron	A/G	0.07	0.880	OPA	
	rs3021094	intron	A/C	0.08	1.000	OPA	
	rs1800871	flanking 5′UTR	G/A	0.23	1.000	OPA	rs1800872
	rs1800894	flanking 5′UTR	G/A	0.04	1.000	KBIO	
	rs1800896	flanking 5′UTR	A/G	0.50	0.039	OPA	rs3024496
	rs1800890	flanking 5′UTR	T/A	0.39	0.009	OPA	
IL10RA (11q23)	rs4252301	SYNON	G/C	0.11	1.000	OPA	
	rs4252253	intron	C/A	0.33	0.942	OPA	
	rs2256111	SYNON	G/A	0.17	0.655	KBIO	
	rs3135932	NONSYN (Ser/Gly)	A/G	0.11	1.000	KBIO	
	rs4252279	intron	G/A	0.03	0.718	OPA	rs4252246
	rs4252314	intron	A/G	0.03	0.627	OPA	
	rs4252286	intron	G/A	0.32	0.935	TAQMAN	
	rs2229113	NONSYN (Gly/Arg)	G/A	0.05	0.253	TAQMAN	rs2512143, rs2512148
	rs2229114	NONSYN (Gly/Arg)	G/A	0.45	1.000	OPA	
	rs9610	3′UTR	G/A	0.32	0.397	KBIO	
IL10RB (21q22)	rs999788	flanking 5′UTR	G/A	0.28	0.970	OPA	
	rs2239573	intron	A/G	0.26	0.537	KBIO	
	rs2834167	NONSYN (Lys/Glu)	A/G	0.22	0.107	OPA	
	rs2284552	intron	C/A	0.45	0.376	OPA	
	rs2849997	intron	C/T	0.12	0.696	KBIO	
	rs8178474	intron	A/G	0.32	0.679	OPA	
	rs2834168	intron	A/G	0.45	0.733	OPA	
	rs2247526	intron	A/G	0.45	0.952	OPA	rs2843701, rs765429
	rs2248118	intron	G/A	0.33	0.951	OPA	

HUGO gene name (region)	dbSNP reference sequence number	SNP context (amino acid change)[1]	Alleles (major/minor)	Minor allele frequency in controls	HWE p-value[2]	Genotyping method[3]	SNPs in high LD[4]
	rs2843944	intron	G/A	0.47	0.966	OPA	
	rs999259	intron	G/A	0.06	0.339	OPA	
	rs8178556	intron	A/C	0.39	0.329	OPA	
	rs1058867	3′UTR	A/G	0.20	0.103	OPA	rs3171425
	rs7281762	flanking 3′UTR	G/A	0.19	0.127	OPA	rs81878562
IL12A (3q25)	rs582537	intron	C/A	0.45	0.652	OPA	rs2227314
	rs2243135	intron	C/G	0.40	0.431	OPA	
	rs2243154	flanking 3′UTR	G/A	0.09	0.972	OPA	
IL12B (5q31)	rs6870828	flanking 3′UTR	T/C	0.48	0.600	KBIO	
	rs3181225	flanking 3′UTR	G/A	0.17	0.995	OPA	rs2569254
	rs3181224	flanking 3′UTR	A/G	0.11	1.000	OPA	
	rs1368439	3′UTR	A/C	0.19	0.624	OPA	
	rs3212227	3′UTR	A/C	0.20	1.000	OPA	rs3212220
	rs3213119	NONSYN (Val/Phe)	C/A	0.03	1.000	OPA	
	rs2853694	intron	A/C	0.48	0.037	KBIO	
	rs2569253	intron	C/T	0.47	0.001	TAQMAN	
	rs3181216	intron	T/A	0.33	0.266	OPA	
	rs1433048	intron	A/G	0.18	0.855	OPA	
	rs2546893	intron	G/A	0.50	0.636	OPA	
	rs730691	intron	G/A	0.37	0.220	OPA	
	rs2546890	flanking 5′UTR	A/G	0.48	0.079	OPA	
	rs10052709	flanking 5′UTR	G/C	0.13	0.745	OPA	
	rs10045431	flanking 5′UTR	C/A	0.29	0.481	OPA	
IL12RB1 (19p13)	rs445521	flanking 3′UTR	A/C	0.42	0.740	OPA	
	rs17885963	flanking 3′UTR	GC	0.05	1.000	OPA	
	rs3746190	3′UTR	G/A	0.39	0.600	OPA	
	rs383483	intron	A/G	0.48	0.195	OPA	
	rs438421	intron	G/A	0.28	0.339	OPA	
	rs2045386	intron	G/A	0.42	0.872	OPA	
	rs11575934	NONSYN (Gln/Arg)	A/G	0.32	0.293	TAQMAN	rs375947, rs391410, rs17852635

HUGO gene name (region)	dbSNP reference sequence number	SNP context (amino acid change)[1]	Alleles (major/minor)	Minor allele frequency in controls	HWE p-value[2]	Genotyping method[3]	SNPs in high LD[4]
	rs11575926	NONSYN (Arg/His)	G/A	0.17	0.638	KBIO	
	rs11673460	intron	G/T	0.06	1.000	KBIO	
	rs11086087	SYNON	G/C	0.12	0.194	OPA	rs17879591, rs3761041
IL12RB2 (1p31)	rs3762315	flanking 5′UTR	A/G	0.20	0.000	OPA	
	rs3762317	flanking 5′UTR	A/G	0.15	0.409	OPA	
	rs12142823	5′UTR	C/T	0.16	1.000	TAQMAN	
	rs7544381	intron	G/A	0.36	0.914	OPA	
	rs17129741	intron	TA	0.04	0.506	OPA	rs2030071
	rs12138219	intron	A/G	0.20	0.846	OPA	
	rs17129794	intron	A/C	0.17	0.400	OPA	
	rs1495963	SYNON	G/A	0.01	0.328	OPA	
	rs12145984	intron	G/C	0.17	0.766	OPA	
	rs3790565	intron	A/G	0.18	0.322	OPA	rs6660836
	rs10749775	intron	A/C	0.12	0.064	OPA	
	rs3790567	intron	G/A	0.24	0.776	OPA	rs3977726
	rs2307153	NONSYN (Gly/Asp)	G/A	0.02	1.000	OPA	
	rs17129908	intron	A/T	0.12	0.092	OPA	
	rs4297265	SYNON	A/G	0.43	0.862	OPA	rs881086
	rs2229546	SYNON	A/C	0.34	0.215	TAQMAN	rs6699474, rs7555183

[1] Location of polymorphism within gene: flanking 5′UTR is upstream of the first exon of the gene, 5′ UTR is in the upstream untranslated region, 3′ flanking is downstream of the last exon of the gene, 3′ UTR is in the downstream untranslated region, exon is in coding region of the gene, intron is between coding regions of the gene. All SNPs are listed in the 5′ to 3′ direction on the forward strand

[2] Pearson's chi-square p-value for compliance with Hardy-Weinberg equilibrium

[3] OPA = Illumina GoldenGate Custom Assay, KBIO = KASPar SNP Genotyping Assays, TAQMAN = TaqMan Probe Applied or Custom SNP Genotyping Assays

[4] SNPs listed in this column represent SNPs that were genotyped in cases, parents, and population controls in this study and found to be in high linkage disequilibrium (LD), defined as $r^2 \geq 0.80$, and were therfore excluded from further analyses

Table 3

IL10, IL12A, IL12B, IL10RA, IL10RB, IL12RB1, and *IL12RB2* tagSNP associations with cervical and vulvar cancer, Seattle-Puget Sound region, 1986–2004

Gene	SNP	Cervical squamous cell carcinoma				Cervical adenocarcinoma				Vulvar squamous cell carcinoma				MAF in population controls
		16 triads, 27 dyads, 357 unrelated cases, and 861 population controls				55 triads, 41 dyads, 406 unrelated cases, and 861 population controls				17 triads, 36 dyads, 474 unrelated cases, and 1,111 population controls				
		MAF[1] (cases)	Odds ratio	95% CI	Holm-adjusted p-value	MAF (cases)	Odds ratio	95% CI	Holm-adjusted p-value	MAF (cases)	Odds ratio	95% CI	Holm-adjusted p-value	
IL10	rs3024505	0.14	0.70	(0.37,1.36)		0.14	1.21	(0.8,1.82)		0.16	0.57	(0.27,1.17)		0.15
	rs3024498	0.26	1.31	(0.82,2.08)		0.26	0.90	(0.63,1.27)		0.25	0.89	(0.54,1.45)		0.27
	rs3024509	0.07	1.09	(0.48,2.48)		0.07	0.78	(0.4,1.52)		0.06	1.64	(0.84,3.19)		0.07
	rs3021094	0.07	0.45	(0.15,1.35)		0.08	1.11	(0.64,1.9)		0.08	1.28	(0.66,2.48)		0.08
	rs1800871	0.23	1.11	(0.65,1.89)		0.24	1.10	(0.79,1.53)		0.25	1.62	(1.04,2.54)	0.060	0.23
	rs1800894	0.04	0.86	(0.27,2.79)		0.03	0.51	(0.17,1.58)		0.03	1.16	(0.42,3.25)		0.04
	rs1800896	0.46	1.22	(0.79,1.89)		0.48	1.00	(0.75,1.33)		0.46	0.91	(0.58,1.43)		0.50
	rs1800890	0.38	1.26	(0.81,1.96)		0.36	1.10	(0.82,1.47)		0.37	0.78	(0.53,1.15)		0.39
IL10RA	rs4252301	0.09	0.57	(0.03,9.55)		0.11	1.05	(0.25,4.34)		0.11	NE			0.11
	rs4252253	0.33	0.38	(0.16,0.91)	0.100	0.31	1.13	(0.74,1.71)		0.38	1.02	(0.58,1.79)		0.33
	rs2256111	0.16	0.67	(0.4,1.12)		0.15	1.21	(0.88,1.68)		0.19	0.95	(0.61,1.48)		0.17
	rs3135932	0.10	1.11	(0.56,2.17)		0.11	1.21	(0.8,1.83)		0.11	0.79	(0.41,1.51)		0.11
	rs4252279	0.04	0.64	(0.25,1.61)		0.04	1.02	(0.61,1.7)		0.04	0.95	(0.45,1.99)		0.03
	rs4252314	0.02	1.57	(0.63,3.93)		0.03	2.23	(1.26,3.96)	0.002	0.02	1.11	(0.33,3.72)		0.03
	rs4252286	0.31	0.52	(0.1,2.74)		0.31	0.71	(0.24,2.08)		0.36	0.28	(0.03,2.59)		0.32
	rs2229113	0.05	1.01	(0.62,1.67)		0.05	1.16	(0.84,1.6)		0.05	0.95	(0.58,1.56)		0.05
	rs2229114	0.46	0.48	(0.11,2.07)		0.46	1.03	(0.48,2.22)		0.41	1.92	(0.88,4.15)		0.45
	rs9610	0.33	1.76	(1.15,2.68)	0.001	0.31	0.75	(0.53,1.06)		0.37	1.16	(0.72,1.87)		0.32
IL10RB	rs999788	0.30	0.90	(0.5,1.61)		0.29	0.94	(0.63,1.41)		0.28	0.72	(0.39,1.31)		0.28
	rs2239573	0.25	1.12	(0.68,1.83)		0.26	1.07	(0.78,1.47)		0.25	1.13	(0.71,1.81)		0.26
	rs2834167	0.20	0.97	(0.54,1.75)		0.22	0.97	(0.68,1.39)		0.21	0.70	(0.42,1.18)		0.22
	rs2284552	0.44	0.85	(0.45,1.61)		0.43	0.96	(0.66,1.41)		0.46	0.75	(0.42,1.34)		0.45
	rs2849997	0.11	1.02	(0.61,1.72)		0.12	0.98	(0.7,1.37)		0.12	0.94	(0.64,1.39)		0.12
	rs8178474	0.30	0.73	(0.34,1.6)		0.30	0.98	(0.61,1.58)		0.30	0.64	(0.32,1.3)		0.32
	rs2834168	0.47	0.70	(0.43,1.15)		0.42	0.98	(0.71,1.34)		0.45	1.05	(0.7,1.58)		0.45
	rs2247526	0.47	1.24	(0.76,2.01)		0.43	0.87	(0.63,1.2)		0.46	1.27	(0.85,1.89)		0.45

Gene	SNP	Cervical squamous cell carcinoma				Cervical adenocarcinoma				Vulvar squamous cell carcinoma				MAF in population controls
		16 triads, 27 dyads, 357 unrelated cases, and 861 population controls				55 triads, 41 dyads, 406 unrelated cases, and 861 population controls				17 triads, 36 dyads, 474 unrelated cases, and 1,111 population controls				
		MAF[1] (cases)	Odds ratio	95% CI	Holm-adjusted p-value	MAF (cases)	Odds ratio	95% CI	Holm-adjusted p-value	MAF (cases)	Odds ratio	95% CI	Holm-adjusted p-value	
	rs2248118	0.30	1.09	(0.69,1.71)		0.33	0.93	(0.66,1.31)		0.31	1.16	(0.8,1.67)		0.33
	rs2843944	0.50	0.50	(0.28,0.91)	0.120	0.48	0.97	(0.7,1.34)		0.46	0.97	(0.62,1.52)		0.47
	rs999259	0.05	1.32	(0.91,1.92)		0.05	0.86	(0.64,1.15)		0.06	1.23	(0.82,1.83)		0.06
	rs8178556	0.38	0.83	(0.28,2.42)		0.38	1.23	(0.69,2.19)		0.39	0.85	(0.28,2.59)		0.39
	rs1058867	0.19	0.89	(0.53,1.47)		0.19	1.06	(0.77,1.46)		0.19	1.15	(0.74,1.77)		0.20
	rs7281762	0.20	0.73	(0.38,1.41)		0.19	1.11	(0.76,1.61)		0.19	0.74	(0.44,1.25)		0.19
IL12A	rs582537	0.42	0.64	(0.38,1.08)		0.44	0.97	(0.72,1.31)		0.46	0.98	(0.65,1.47)		0.45
	rs2243135	0.40	1.17	(0.72,1.91)		0.38	0.94	(0.69,1.29)		0.39	0.86	(0.55,1.35)		0.40
	rs2243154	0.09	1.03	(0.41,2.61)		0.10	0.70	(0.39,1.26)		0.09	1.19	(0.67,2.1)		0.09
IL12B	rs6870828	0.49	1.53	(0.86,2.73)		0.49	1.05	(0.75,1.49)		0.48	0.91	(0.6,1.4)		0.48
	rs3181225	0.20	1.85	(1.07,3.21)	0.080	0.20	1.14	(0.76,1.71)		0.21	1.16	(0.69,1.96)		0.17
	rs3181224	0.11	1.16	(0.58,2.32)		0.11	1.08	(0.63,1.85)		0.09	0.30	(0.12,0.74)	0.001	0.11
	rs1368439	0.17	0.84	(0.41,1.68)		0.19	0.96	(0.64,1.43)		0.18	0.92	(0.55,1.54)		0.19
	rs3212227	0.21	0.50	(0.23,1.09)		0.18	0.87	(0.59,1.29)		0.20	0.98	(0.61,1.58)		0.20
	rs3213119	0.03	0.52	(0.11,2.48)		0.03	0.66	(0.2,2.13)		0.02	0.50	(0.14,1.84)		0.03
	rs2853694	0.49	0.80	(0.49,1.33)		0.49	1.03	(0.75,1.42)		0.49	1.23	(0.81,1.85)		0.48
	rs2569253	0.46	0.64	(0.39,1.04)		0.46	0.93	(0.68,1.28)		0.44	0.69	(0.45,1.07)		0.47
	rs3181216	0.31	0.90	(0.52,1.53)		0.31	1.00	(0.72,1.4)		0.33	1.22	(0.8,1.86)		0.33
	rs1433048	0.17	0.88	(0.45,1.73)		0.22	0.85	(0.58,1.26)		0.18	1.12	(0.67,1.88)		0.18
	rs2546893	0.50	1.23	(0.78,1.93)		0.49	1.12	(0.82,1.52)		0.48	1.09	(0.73,1.63)		0.50
	rs730691	0.36	0.57	(0.32,1.01)	0.450	0.36	0.86	(0.62,1.2)		0.36	0.98	(0.66,1.44)		0.37
	rs2546890	0.48	1.16	(0.75,1.79)		0.47	0.99	(0.74,1.32)		0.47	0.89	(0.58,1.36)		0.48
	rs10052709	0.14	1.11	(0.61,2.03)		0.14	0.96	(0.59,1.57)		0.10	0.67	(0.34,1.32)		0.13
	rs10045431	0.28	0.90	(0.53,1.51)		0.30	1.02	(0.73,1.43)		0.26	0.65	(0.41,1.03)		0.29
IL12RB1	rs445521	0.40	1.08	(0.65,1.79)		0.40	1.00	(0.74,1.34)		0.39	0.98	(0.65,1.49)		0.42
	rs17885963	0.06	2.00	(1.03,3.91)	0.320	0.05	0.70	(0.34,1.48)		0.05	1.07	(0.45,2.53)		0.05
	rs3746190	0.40	1.65	(0.97,2.8)		0.37	1.04	(0.77,1.43)		0.37	0.70	(0.49,1.01)	0.150	0.39
	rs383483	0.50	0.78	(0.47,1.3)		0.50	0.75	(0.55,1.01)	0.150	0.49	1.31	(0.88,1.94)		0.48

Gene	SNP	Cervical squamous cell carcinoma				Cervical adenocarcinoma				Vulvar squamous cell carcinoma				MAF in population controls
		16 triads, 27 dyads, 357 unrelated cases, and 861 population controls				55 triads, 41 dyads, 406 unrelated cases, and 861 population controls				17 triads, 36 dyads, 474 unrelated cases, and 1,111 population controls				
		MAF[1] (cases)	Odds ratio	95% CI	Holm-adjusted p-value	MAF (cases)	Odds ratio	95% CI	Holm-adjusted p-value	MAF (cases)	Odds ratio	95% CI	Holm-adjusted p-value	
	rs438421	0.30	0.77	(0.48,1.24)		0.28	0.82	(0.57,1.17)		0.28	1.03	(0.63,1.68)		0.28
	rs2045386	0.40	1.13	(0.73,1.74)		0.42	0.79	(0.58,1.09)		0.40	0.84	(0.57,1.25)		0.42
	rs11575934	0.32	0.94	(0.56,1.6)		0.32	1.51	(1.12,2.05)	0.001	0.33	1.16	(0.79,1.71)		0.32
	rs11575926	0.18	0.97	(0.51,1.85)		0.16	1.39	(0.97,2.00)		0.18	0.79	(0.48,1.32)		0.17
	rs11673460	0.07	1.59	(0.7,3.62)		0.05	0.72	(0.36,1.42)		0.06	0.64	(0.28,1.46)		0.06
	rs11086087	0.09	0.54	(0.2,1.45)		0.13	1.49	(0.96,2.3)		0.13	1.44	(0.83,2.52)		0.12
IL12RB2	rs3762315	0.18	0.70	(0.38,1.27)		0.22	0.70	(0.46,1.07)		0.18	1.18	(0.75,1.85)		0.20
	rs3762317	0.13	0.71	(0.33,1.52)		0.17	0.70	(0.42,1.15)		0.13	1.18	(0.67,2.09)		0.15
	rs12142823	0.14	0.61	(0.27,1.34)		0.18	0.76	(0.46,1.27)		0.14	1.19	(0.67,2.13)		0.16
	rs7544381	0.33	0.70	(0.41,1.18)		0.36	0.92	(0.66,1.28)		0.34	1.08	(0.7,1.67)		0.36
	rs17129741	0.04	0.71	(0.20,2.59)		0.03	0.66	(0.26,1.63)		0.04	1.16	(0.45,3)		0.04
	rs12138219	0.18	0.64	(0.33,1.25)		0.21	0.70	(0.45,1.1)		0.19	1.31	(0.77,2.22)		0.20
	rs17129794	0.16	0.59	(0.29,1.2)		0.18	1.03	(0.7,1.52)		0.19	0.88	(0.5,1.55)		0.17
	rs1495963	0.02	0.73	(0.13,4.14)		0.02	1.42	(0.51,3.93)		0.02	1.56	(0.48,5.04)		0.01
	rs12145984	0.14	0.47	(0.16,1.39)		0.17	1.02	(0.66,1.58)		0.15	0.70	(0.39,1.24)		0.17
	rs3790565	0.18	0.49	(0.25,0.95)	0.210	0.18	0.89	(0.6,1.31)		0.18	0.86	(0.48,1.55)		0.18
	rs10749775	0.12	0.53	(0.22,1.27)		0.13	1.00	(0.64,1.55)		0.13	0.90	(0.44,1.85)		0.12
	rs3790567	0.23	0.58	(0.32,1.03)	0.600	0.23	0.86	(0.6,1.22)		0.24	0.91	(0.56,1.48)		0.24
	rs2307153	0.02	0.86	(0.17,4.44)		0.02	1.18	(0.48,2.89)		0.03	1.60	(0.44,5.77)		0.02
	rs17129908	0.12	0.66	(0.32,1.38)		0.10	0.79	(0.48,1.33)		0.12	0.86	(0.47,1.59)		0.12
	rs4297265	0.40	0.46	(0.26,0.82)	0.002	0.42	1.01	(0.74,1.37)		0.42	0.84	(0.57,1.23)		0.43
	rs2229546	0.33	0.43	(0.21,0.87)	0.030	0.35	1.12	(0.8,1.57)		0.33	0.86	(0.57,1.31)		0.34

[1]MAF: Minor allele frequency

Table 4

Selected tagSNP associations with cervical cancer, by HPV DNA type detected in tissue

Gene	SNP	HPV 16[1]		HPV 18[1]	
		19 triads, 21 dyads, 331 unrelated cases, and 861 population controls		9 triads, 6 dyads, 171 unrelated cases, and 861 population controls	
		Odds ratio	95% CI	Odds ratio	95% CI
IL10RA	rs4252253	0.84	(0.41, 1.71)	1.98	(0.75, 5.19)
IL10RA	rs4252314	2.73	(1.15, 6.47)	3.92	(1.38, 11.13)
IL10RA	rs9610	1.16	(0.74, 1.82)	0.51	(0.26, 0.99)
IL10RB	rs2843944	0.98	(0.57, 1.68)	0.84	(0.34, 2.03)
IL12B	rs3181225	1.66	(0.95, 2.91)	0.63	(0.20, 1.99)
IL12B	rs730691	0.65	(0.38, 1.12)	1.01	(0.43, 2.38)
IL12RB1	rs17885963	1.26	(0.59, 2.73)	0.92	(0.17, 4.99)
IL12RB1	rs383483	0.69	(0.40, 1.18)	0.88	(0.42, 1.83)
IL12RB1	rs11575934	1.38	(0.85, 2.22)	1.10	(0.56, 2.18)
IL12RB2	rs3790565	0.60	(0.30, 1.20)	1.05	(0.51, 2.18)
IL12RB2	rs3790567	0.62	(0.34, 1.13)	0.72	(0.33, 1.56)
IL12RB2	rs4297265	0.59	(0.36, 0.95)	1.85	(0.90, 3.81)
IL12RB2	rs2229546	0.59	(0.33, 1.06)	2.73	(1.21, 6.13)

[1] Includes cases that were both HPV16 and HPV18 positive