Cervical and Vulvar Cancer Risk in Relation to Joint Effects of Cigarette Smoking and Genetic Variation in Interleukin 2

Shehnaz K. Hussain[1,2], Margaret M. Madeleine[1], Lisa G. Johnson[1], Qin Du[3], Mari Malkki[3], Hui-Wen Wilkerson[4], Federico M. Farin[4], Joseph J. Carter[5], Denise A. Galloway[5], Janet R. Daling[1], Effie W. Petersdorf[3], Stephen M. Schwartz[1]

[1]Program in Epidemiology, Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA. [2]UCLA Division of Cancer Prevention and Control Research, School of Public Health and Jonsson Comprehensive Cancer Center, Los Angeles, CA. [3]Program in Immunogenetics, Division of Clinical Research, Fred Hutchinson Cancer Research Center, Seattle, WA. [4]Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, WA. [5]Program in Cancer Biology, Divisions of Human Biology and Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA.

Running title: Smoking, *IL2* tagSNPs, and cervical and vulvar cancer risk

Key words: Cervical cancer, Vulvar cancer, Effect modification, Interleukin 2, Cigarette smoking

Footnotes:

Financial support: This research was funded by grants from the U.S. National Institutes of Health (R01CA112512; P01CA042792, R25CA094880, P30ES07033).

Correspondence: Requests for reprints can be sent to Shehnaz K. Hussain, UCLA Division of Cancer Prevention and Control Research, School of Public Health and Jonsson Comprehensive Cancer Center, 650 Charles Young Drive South A2-125 CHS, Box 956900, Los Angeles, CA 90095-6900, email: skhussain@ucla.edu

Conflicts of Interest: None of the authors have a conflict of interest to report.

Abstract

Cigarette smoking is an established co-factor to human papillomavirus (HPV) in the development of cervical and vulvar squamous cell carcinoma (SCC), and may influence risk through an immunosuppressive pathway. Genetic variation in interleukin 2 (*IL2*), associated in some studies with inhibition of HPV-targeted immunity, may modify the effect of smoking on the risk of HPV-related anogenital cancers. We conducted a population-based case-only study to measure the departure from a multiplicative joint effect of cigarette smoking and *IL2* variation on cervical and vulvar SCC. Genotyping of four *IL2* tagSNPs (rs2069762, rs2069763, rs2069777, and rs2069778) was performed in 399 cervical and 486 vulvar SCC cases who had been interviewed regarding their smoking history. Compared to cases carrying the rs2069762 TT genotype, we observed significant departures from multiplicativity for smoking and carriership of the TG or GG genotypes in vulvar SCC risk (interaction odds ratio (IOR)=1.67, 95% confidence interval (CI): 1.16, 2.41). Carriership of one of three diplotypes together with cigarette smoking was associated with either a supra-multiplicative (TGCT/GGCC, IOR=2.09, 95% CI: 0.98, 4.46) or sub-multiplicative (TTCC/TGTC, IOR=0.37, 95% CI: 0.16, 0.85 or TGCT/TGCC, IOR=0.37, 95% CI: 0.15, 0.87) joint effect in vulvar cancer risk. For cervical SCC, departure from multiplicativity was observed for smokers homozygous for the rs2069763 variant allele (TT versus GG or GT genotypes) (IOR=1.87, 95% CI: 1.00, 3.48), and for carriership of the TTCC/TTCC diplotype, (IOR=2.08, 95% CI: 1.01, 4.30). These results suggest that cervical and vulvar SCC risk among cigarette smokers is modified by genetic variation in *IL2*.

Introduction

Persistent oncogenic human papillomavirus (HPV) infection is etiologically linked to all cervical cancers and a large subset of vulvar cancers (1). The HPV-dependent vulvar cancers are associated with nonkeratinizing basaloid or warty vulvar intraepithelial neoplasia and primarily affect younger women. They bear remarkable resemblance to cervical squamous intraepithelial neoplasia and cancer, and are associated with similar HPV types and co-factors (2, 3).

Cigarette smoking is among the most well-established HPV-co-factors in the etiology of these malignancies (4). Current smokers are at approximately two- to three-fold increased cervical squamous cell carcinoma (SCC) risk (5), and greater than three-fold vulvar SCC risk (2, 3), whereas former cigarette smokers tend to be at little or no increased risk (5, 6). Studies have also observed an association of cervical SCC risk with increasing duration of smoking (5, 7), although this trend appears to be driven by the high proportion of long-term smokers who are also current smokers (7). Experimental evidence linking smoking cessation and a decrease in cervical lesion size (8) also highlights the important role of current cigarette smoking in cervical SCC risk.

The biological mechanism whereby cigarette smoking increases cervical and vulvar SCC risk remains largely undetermined (9). One possibility is that smoking enhances immunosuppression (8). The importance of the adaptive immune response in HPV-associated cancer risk is emphasized by studies showing that HIV-infected women have a substantially increased risk of developing cervical and vulvar cancer (10, 11), and women with drug-induced immunosuppression are nine times more likely than the general population to develop an HPV infection, and 16 times more likely to develop cervical cancer (12). In immunocompetent patients capable of preventing persistent HPV infection and related neoplastic changes, Th1 cytokines such as interleukin 2 (IL-2) propagate a T lymphocyte-mediated immune response to HPV and tumor antigens (13-16). IL-2 is a T lymphocyte derived

cytokine that is secreted minutes after activation of a T lymphocyte receptor by an antigen bound to a major histocompatibility complex receptor on an antigen presenting cell. IL-2 acts in an autocrine manner by binding the IL-2 receptor on activated T lymphocytes and inducing transcription of other Th1 cytokines, which together propagate the T lymphocyte response (17). IL-2 is considered to be a key component of the adaptive immune response to HPV infection and the development and growth of tumors driven by the viral oncogenes (18, 19).

Experimental studies demonstrate an influence of both cigarette smoking (20-24) and genetic variation (25) on IL-2 expression, suggesting the possibility that cigarette smoking and inherited genetic variation in *IL2* interact to increase cervical and vulvar SCC risk. We conducted the present study to test that hypothesis.

Methods

Study design

Assessing the joint effect of cigarette smoking and *IL2* nucleotide variation on HPV-dependent cancers would ideally involve assessing the interaction effect among women who have persistent oncogenic HPV infection (26). Practically, however, oncogenic HPV infection in the general population of adult women identified with current detection methods is uncommon (between 2 and 12%), and persistent infection is rare (27). A case-only design avoids the difficult task of selecting a control group with persistent HPV infection. Under the assumption of independence between cigarette smoking and variation in *IL2*, the interaction odds ratio (IOR) from a case-only design provides an estimate of effect modification equivalent to that derived from a case-control study under a multiplicative model (28). In addition, the case-only design offers higher precision to estimate the IOR compared to a standard case-control design (29).

Study population

This study was ancillary to a large population-based case-control study focused on host and environmental factors that contribute to HPV-related anogenital cancer risk (2, 30). Briefly, the case-control study attempted to recruit all 18 to 74 year-old residents of King, Pierce, and Snohomish counties, Washington, diagnosed with incident invasive cervical and invasive or *in situ* vulvar cancer 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 (SEER) program (31). To help ensure comparability between the cases and controls, who were identified and recruited using a one-step modification of the Waksberg-Mitofsky method of random-digit telephone dialing (32, 33) and frequency matched to cases by five-year age groups, only cases with residential telephones were eligible for the study. Cases with tumors that were not SCC (e.g., adenocarcinoma) were excluded from this ancillary study as those histologies are not related to cigarette smoking. Non-Caucasian women were excluded from this study because they comprised less than 10% of the original study population, precluding meaningful sub-group analyses stratified by race while increasing the possibility of bias due to population stratification. A sample of Caucasian controls from the parent study was included in this "case-only" study to test the assumption of independence between genotypes of *IL2* variants and cigarette smoking. The cervical cancer control group was restricted to women without prior hysterectomy, thus reflecting the population from which the cases arose. No such restrictions were placed on the vulvar cancer controls

Data and specimen collection

In the case-control study, in-person interviews were conducted to elicit information on demographic and other characteristics with a known or suspected relationship to anogenital cancer, including cigarette smoking. A woman was considered a smoker if she reported smoking 100 or more cigarettes in her lifetime. Venous blood samples were drawn at the time of the interview to provide serum samples for HPV 16 and 18 antibody testing as described previously (34). Beginning in 1991, five years after the start of the study, we expanded the blood collection to include samples from which DNA could be isolated. We also recontacted cervical, but not vulvar, cancer cases interviewed in the earliest years of the study and asked them to provide these additional blood samples. A small proportion of study participants (3%) preferred to donate a buccal cell sample, which was collected using a standardized oral rinse procedure, in place of blood. We attempted to retrieve archival tissue blocks from biopsy or surgery to determine the presence and type of HPV DNA in the tumors of the cervical and vulvar cancer cases. HPV DNA typing on tumor tissue was performed using polymerase chain reaction (PCR) methods, as described in detail previously (35).

Response Rates

Among the 1,189 eligible cervical SCC patients identified for the parent case-control study, 744 (62.6%) were interviewed and among those interviewed 674 (90.6%) provided a specimen from which DNA could be obtained. A similar proportion, 67.6%, (807 of the 1194 eligible vulvar SCC cases) were interviewed, however, specimens from which DNA could be obtained were only collected from 73.4% of participating vulvar cancer cases. This percentage is largely affected by the fact that, as described above, the early version of the parent study protocol did not include collection of blood specimens from which DNA could be isolated, and that and the vulvar cancer cases, unlike the cervical cancer cases, were not reapproached for these specimens once the protocol was changed. Reasons for

non-participation were largely similar for the two cancers and included doctor refusal to allow us to contact the patient (5% and 6%, for cervical and vulvar cases respectively), refusal of the patient to participate or our inability to locate the patient (22% and 24%), or patient death (10% and 3%). Drawn from the Caucasian participants who had a sufficient DNA sample at the time of this study, our analyses included 399 cervical and 490 (434 *in situ*) vulvar SCC cases. Four vulvar SCC cases (3 *in situ*) were not included in any of the tables because a genotyping result could not be obtained from their samples for any of the polymorphisms included in this study, resulting in a total of 486 vulvar SCC cases. Sixty-three % (n=251) of cervical cancer cases and 71% (n=347) of vulvar cancer cases included in this study had tumor tissue available that had been tested for HPV DNA. Sixty-seven % of eligible control women agreed to participate, and 83.9% (N=1,372) of those interviewed donated a blood sample from which DNA could be obtained.

The parent population-based study had no measure of HPV DNA in the cervix or vulva for control subjects. Yet, the assessment of independence of cigarette smoking and *IL2* genotypes is best in a control sample that comes from the same pool of HPV infected women that give rise to the cases in this study. Thus, among the 1,094 eligible controls with genomic DNA available, we included in the present study only those that were positive for HPV16 or HPV18 L1 serum antibodies, a measure of past exposure to the virus, by a virus-like particle assay (n=236) (34).

TagSNP selection

Information on *IL2* nucleotide variation was obtained from the SeattleSNPs Variation Discovery Resource (36), http://pga.gs.washington.edu/data/il2/. Briefly, SeattleSNPs has resequenced exons, introns, and 1000 bp or more on the 5' and 3' ends of each target gene in DNA from 23 Centre d'Etude du Polymorphisme Humain (CEPH) parents of European descent and 24 African-Americans,

obtained from the Coriell Repository (Camden, NJ). Using the European descent data, all SNPs with a variant allele frequency of at least 5% were identified; seven out of the ten SNPs met this criterion. Next, a pairwise r^2 cutpoint of 0.80 was used to delineate groups of highly correlated SNPs (37) and one polymorphism (i.e. tagSNPs) per group was selected to be genotyped. When more than one possible tagSNP for a particular group of correlated SNPs was identified, information regarding putative function reported in the literature and location of the SNP informed tagSNP selection. The National Center for Bioinformatics (NCBI) dbSNP build 127 reference sequence number for the four selected *IL2* tagSNPs are rs2069762, rs2069763, rs2069777, and rs2069778.

Genotyping of IL2 tagSNPs

Genomic DNA was extracted from buffy coat aliquots from blood samples, or cell pellets from buccal samples, using a phenol chloroform method (38). Genotyping was performed using Pre-Designed or Custom TaqMan® genotyping assays from Applied Biosystems following manufacturer's protocol (Applied Biosystems, Foster City, CA). Briefly, the assays were conducted in a 5 μl volume containing 5 to 50 ng genomic DNA, 2.5 μl of the 2x Universal Master Mix with uracil-DNA glucosylase 200 nM of each assay-specific primer and 900 nM of each assay-specific FAM and VIC fluorescently labeled probe. Reactions were amplified using a 9700 PCR machine or a 7500 Real-Time PCR system (Applied Biosystems, Foster City, CA) for 50ºC for 2 min, 95ºC 10 min followed by 40 to 50 cycles of 92ºC for 15 to 30 s and 58 to 60ºC for 1 to 1.5 min. The fluorescence release was measured by the 7500 Real-Time PCR system using the allelic discrimination setting of the Sequence Detection Software version 1.2.3 (Applied Biosystems). Probe and primer sequences are listed in Supplementary Table 1. Two to three positive controls (samples known to be heterozygous or

homozygous for each allele based on sequencing) and negative controls (wells containing no DNA) were included in each reaction plate. Specimens were organized so that the replicate QC DNA aliquots, which comprised approximately 10% of the specimens, were distributed throughout the reaction plates. Analysis of these replicates revealed a low discordance proportion of 1%. Laboratory personnel were blinded to all research information about the samples, including the identities of the QC replicate aliquots.

Data analysis

TagSNP genotypes were tested for consistency with Hardy-Weinberg equilibrium (HWE) within the HPV seropositive control sample using a Pearson's χ^2 *p* value cutpoint of 0.05. The control sample was also used to test for independence of smoking status and *IL2* tagSNP genotypes. One approach to test for independence is to use logistic regression to model smoking as a dependent variable and genotype as an independent variable among the controls. Alternatively, Umbach and Weinberg (1997) proposed a method which offers higher precision that uses a likelihood ratio test (LRT) to compare two nested log-linear models for each tagSNP (39). In the full model, the logarithm of the expected cell count was the dependent variable that fully parameterizes the joint effect of cigarette smoking and tagSNP genotypes separately for cases and controls. The reduced model fixed the joint effect parameter for the controls at zero. Thus the LRT comparing these two models is a test of the association between tagSNP genotypes and cigarette smoking among controls. An LRT *p* value of 0.05 or less, or an exponentiated joint effect parameter for cigarette smoking and tagSNP genotype among controls (The OR from the full model) departing substantially from the null, was taken as evidence of a statistically significant lack of independence between cigarette smoking status and *IL2*

genotypes. For the cervical cancer analyses, these models were fit after excluding 56 controls without intact uteri, resulting in 180 controls.

For tagSNPs that met the independence criteria, IORs and 95% confidence intervals (CIs) were calculated using logistic regression. Separately for the cervical and vulvar cancer case groups, current cigarette smokers were compared to former or never smokers as the outcome variable, and tagSNP genotypes comprised the predictor variables. The IORs represent the departure of the joint effect of *IL2* tagSNP genotypes and current cigarette smoking from that expected under a multiplicative model, on cervical and vulvar cancer risk. Genotype IORs were calculated without restricting to a particular genetic model, and additional IORs were calculated assuming dominant and recessive penetrance. Genotype IORs were also calculated on the sub-group of vulvar cancer cases testing positive for oncogenic HPV DNA in their tumors or positive for HPV16 or HPV18 L1 serum antibodies (n=325). Age at diagnosis, tumor stage, education, number of lifetime sexual partners, parity, oral contraceptive use, and family history of anogenital cancer were considered as potential confounding factors of the IORs, but did not have substantial influences and were not included in the final models.

PHASE version 2.1 software (40) was used to statistically infer haplotypes in *IL2*. A log-additive genetic model was assumed to obtain haplotype IORs and 95% CIs using logistic regression. We accounted for some of the uncertainty inherent in statistical determination of haplotypes by including all PHASE-inferred haplotypes into our logistic regression models as separate observations, weighted in proportion to their PHASE-inferred probabilities of being the true haplotype (41). We also calculated IORs and 95% CIs for pairs of haplotypes (diplotypes) using similar weighted logistic regression models. In the sections that follow, SNP alleles in each haplotype are listed from 5' to 3’ (rs2069762, rs2069763, rs2069777, rs2069778), and the variant allele at each locus is underlined.

The main effect of each tagSNP on cervical and vulvar cancer risk was assessed. Cervical cancer cases and vulvar cancer cases were compared to HPV16 or HPV18 L1 seropositive controls, and sub-analyses were conducted in which oncogenic HPV DNA positive or HPV16 or HPV18 L1 seropositive vulvar cancer cases were compared to HPV16 or HPV18 L1 seropositive controls. Cervical cancer analyses were conducted after excluding controls without intact uteri. Separate logistic regression models were used to estimate genotype-specific ORs and 95% CI for each tagSNP and cancer site.

Results

Selected characteristics of the cervical and vulvar cancer cases included in this study are presented in Table 1. Eighty-nine % of the vulvar cancer cases in this study were diagnosed with *in situ* tumors, and 83% of the cervical cancer cases were diagnosed with an invasive tumor staged (FIGO) 2b or less. On average, the vulvar and cervical cancer case groups were similar with respect to HPV positivity, education level and oral contraceptive usage. However, the vulvar cancer cases were older, more likely to be current smokers, had more sexual partners, had fewer live births, and were more likely to have had a family history of anogenital cancer compared to cervical cancer cases.

TagSNP variant allele frequencies ranged from 0.07 to 0.38 (Table 2). We did not find statistical evidence of lack of fit to HWE for any of the tagSNPs. We observed independence of tagSNP genotypes and cigarette smoking among both cervical and vulvar HPV seropositive control groups, as indicated by ORs close to the null value and LRT p values ≥ 0.05 (Table 2).

Compared to homozygous carriers of the common allele of tagSNP rs2069762 (TT genotype), positive departures from multiplicativity were observed for vulvar cancer cases carrying one (IOR=1.69, 95% CI: 1.15, 2.47), or two (IOR=1.59, 95% CI: 0.76, 3.32) copies of the variant G allele.

The dominant genetic model showed a similar departure for smokers carrying either the TG or GG genotypes, versus carriers of the TT genotype (IOR=1.67, 95% CI: 1.16, 2.41). A similar departure from multiplicativity was observed when the analysis was restricted to the oncogenic HPV DNA positive or HPV16 or HPV18 L1 seropositive vulvar cancer cases, TG or GG genotypes versus TT, IOR=1.92, 95% CI: 1.21, 3.04. However, a slightly increased IOR was observed for all women who were tested for either tumor HPV DNA or HPV serology (N=363, IOR=1.83, 95% CI: 1.20, 2.79) compared to women who did not have tumor tissue available for testing (N=123, IOR=1.25, 95% CI: 0.59, 2.67).

In the recessive genetic model, homozygosity for the variant allele of rs2069763 (TT genotype) and cigarette smoking was associated with a significant positive departure from multiplicativity in cervical cancer risk (IOR=1.87, 95% CI: 1.00, 3.48), which was not observed for vulvar cancer (IOR=0.99, 95% CI: 0.50, 1.94). Genotypes of rs2069777 and rs2069778 did not show elevated or reduced IORs with cigarette smoking in either cervical or vulvar cancer risk.

We observed five haplotypes in *IL2*, each uniquely tagged by the presence of a single variant allele, TTCC, GGCC, TGCT, and TGTC, or no variant alleles, TGCC (as indicated by the underlined allele), Table 4. Compared to carriers of the most common haplotype, TTCC, cigarette smoking and carriership of any other haplotype did not result in significant departures from multiplicativity in either cervical or vulvar cancer risk. The GGCC haplotype, defined by the variant allele of rs2069762, was associated with a positive, but not statistically significant, departure from multiplicativity in vulvar cancer risk (IOR=1.34, 95% CI: 0.94, 1.92). Compared to carriers of the most common diplotype (TTCC/GGCC), carriership of the TTCC/TTCC diplotype, defined by two copies of the variant allele of rs2069763, and cigarette smoking together resulted in a positive departure from multiplicative joint effects on cervical (IOR=2.08, 95% CI: 1.01, 4.30), but not vulvar (IOR=0.85, 95% CI: 0.41, 1.78),

cancer risk (Table 5). The second most common diplotype among smokers with cervical cancer, TTCC/TTCC, defined by two copies of the variant allele of rs2069763, together with cigarette smoking was associated with a significant positive two-fold departure from multiplicatively in cervical cancer risk (IOR=2.08, 95% CI: 1.01, 4.30), compared to the reference diplotype, TTCC/GGCC. Similarly, TGCT/GGCC, a common diplotype among vulvar cancer cases, was associated with a marginally significant positive two-fold departure from multiplicatively in vulvar cancer risk (IOR=2.09, 95% CI: 0.98, 4.46). Two rare diplotypes were associated with sub-multiplicative joint effects in vulvar cancer risk, TTCC/TGTC, IOR=0.37, 95% CI: 0.16, 0.85, and TGCT/TGCC, IOR=0.37, 95% CI: 0.15, 0.87.

The ORs for the main effect of each tagSNP on cervical and vulvar cancer risk are presented in Table 6. Compared to the rs2069762 TT genotype, the TG genotype was associated with a marginally significant increased risk of vulvar cancer (OR=1.28, 95% CI: 0.92-1.78), which was slightly more pronounced when the analysis was restricted to HPV positive vulvar cancer cases (OR=1.42, 95% CI: 1.00-2.03). Compared to the rs2069763 GG genotype, the TT genotype was associated with a statistically significant decreased risk of vulvar cancer (OR=0.45, 95% CI: 0.27-0.76) that was essentially the same when the analysis was restricted to HPV positive cases, and a marginally significant decreased risk of cervical cancer (OR=0.60, 95% CI: 0.35-1.04). The ORs for cervical or vulvar cancer did not deviate significantly from the null for any of the other tagSNPs, nor were there substantial differences in ORs when the analyses were restricted to HPV positive vulvar cancer cases.

Discussion

Cigarette smoking is clearly an important risk factor for cervical and vulvar SCCs, but the mechanism underlying the association is unknown. To our knowledge, this is the first investigation

into effect modification of cigarette smoking by genetic variation in a T lymphocyte regulatory cytokine as a pathway to explain part of the increased risk.

Prior studies have observed the presence of nicotine, cotinine, and other constituents of cigarette smoke and their metabolites in the cervical mucus of smokers (42, 43). These components have been shown to depress populations of cervical Langerhans cells and T lymphocytes (43, 44); cells that both produce and bind IL-2. IL-2 plays a critical role in propagating a Th1 mediated immune response, which is key in combating genital HPV infection and associated neoplasms (13-16). Furthermore, smokers have a near two-fold decrease in IL-2 concentration in cervical secretions, compared to non-smokers (45). Studies of non cervical-derived T lymphocytes have found that components of cigarette smoke, such as nicotine and hydroquinone, inhibit IL-2 production (20-24). Genetic variation in *IL2* may have subtle effects on IL-2 transcription or protein structure that could influence concentrations or receptor binding (25), and potentially these phenotypes could be exacerbated when IL2 production is impaired by smoking. The joint effect of genetic variation and cigarette smoking could conceivably influence the ability of IL-2 to function normally, thereby increasing cancer risk.

In our study, the joint effect of the G allele of tagSNP rs2069762 and cigarette smoking on vulvar cancer risk was nearly two-fold greater than expected under the multiplicative model. While there was a suggestion of an increased vulvar cancer risk associated with heterozygosity for rs2069762, the possibility that this was a spurious finding is supported by observation of a reduced risk of similar magnitude associated with homozygosity for the G allele. The haplotype containing the variant allele of rs2069762, GGCC, was also associated with a supra-multiplicative joint effect with smoking. The IOR for the diplotype carrying two copies of the variant allele of rs2069762, GGCC/GGCC, compared to the reference diplotype which had one copy of the variant allele,

317 TTCC/GGCC, was nearly null. This result is consistent with the single locus model which suggested a
318 dominant genetic effect (i.e. similar IORs for heterozygotes and homozygotes for the variant allele).
319 The rs2069762 polymorphism is located in a 5' flanking, evolutionarily conserved, region of *IL2* (46,
320 47), and the variant allele has been associated with increased IL-2 transcription in cultured peripheral
321 blood lymphocytes (25). Based on these limited experimental data, one might expect carriers of the
322 variant allele (putative high IL-2 producers) to have a stronger T lymphocyte mediated immune
323 response, and thus decreased risk of HPV-related cancer, and in combination with smoking, either no
324 multiplicative effect on risk of HPV-related cancers, or potentially even a sub-multiplicative effect.
325 Alternatively, the putative high IL-2-producing variant allele of rs2069762 may contribute to a positive
326 interaction with cigarette smoking in vulvar cancer risk through an inflammatory pathway. A positive
327 association between inflammation and vulvar cancer risk has been shown previously (48), and the
328 high-producer IL-2 genotype could conceivably lead to an unregulated and unfavorable inflammatory
329 response to HPV infection in vulvar tissue when coupled with cigarette smoking (49). The putative
330 dampening effect of cigarette smoke on IL-2 levels may be outweighed by the tumor promoting
331 potential of cigarette smoking which has been linked to the induction of the pro-inflammatory
332 transcription factor NF-KB (50) and inhibition of apoptosis (51). Thus, while no consistent main effect
333 of the rs2069762 was observed, it is conceivable the joint effect of rs2069762 and cigarette smoking
334 would be important in vulvar cancer risk.
335 Our observation that the joint effect of rs2069762 and cigarette smoking was associated with a
336 positive departure from multiplicativity in vulvar, but not cervical, cancer risk has no obvious
337 explanation. However, as previously mentioned, functional effects of cigarette smoking and genetic
338 variation on IL-2 concentrations have mostly been identified in healthy cervical tissue or peripheral
339 blood, and thus may not reflect the immune environment in vulvar tissue. Unfortunately, there are

limited comparable data on cervical and vulvar HPV or cancer immunity. A few studies suggest that women with cervical and vulvar high grade lesions elicit a similar T lymphocyte responses to HPV (52, 53). In contrast, a study of HPV-16 positive high grade vulvar lesions and cervical cancer reported site-specific associations with polymorphisms of class I and II human leukocyte antigens (HLA) (54), loci that play an important role in regulating T lymphocyte responses to viral proteins. Among the cases and HPV seropositive controls included in this current study, the age-, sex partner-, parity-, and education-adjusted OR for current smoking in cervical cancer risk was 1.48 (95% CI: 0.99-2.22); in vulvar cancer the OR was 3.97 (95% CI: 2.73-5.79). These data, together with prior observations that cervical and vulvar cancer differ in strength of association with cigarette smoking (2-5), suggest that the mechanism of smoking related carcinogenesis may differ between sites. Furthermore, the proportion of current smokers who were heavy smokers (≥1 pack per day) was similar for cervical cancer (64%) and vulvar cancer (60%) cases, and restricting the analyses for rs2069762 to heavy smokers did not substantially influence the IORs. These data add further support to the notion that there may be biological, possibly immunological, differences between the two sites that influence smoking-related carcinogenesis, not simply differences in smoking habits. Lastly, the observed statistically significant joint effect of rs2069762 and cigarette smoking in vulvar cancer risk may be a false positive finding.

Rs2069762 was not in linkage disequilibrium with any other *IL2* SNPs among Caucasians in the SeattleSNPs project, which reduces but does not eliminate the possibility that the interaction we observed was due to linkage with other loci. In the greater 40 kilo-bp region encompassing *IL2*, the International HapMap Project (55) shows linkage between the *IL2* rs2069762 polymorphism and three 3' flanking polymorphisms in the testis nuclear RNA-binding protein gene (TENR, rs716501, rs17454584, and rs4833826), approximately 20 kilo-bp 3' of *IL2*. Little is known regarding tissue-

specific expression of TENR in humans, however in mice TENR is exclusively expressed in the testis thus an influence of these polymorphisms on vulvar cancer risk is highly unlikely (56).

Carriership of two copies of the variant allele of rs2069763, a synonymous SNP, was associated with at least a 1.66-fold excess joint effect with cigarette smoking in cervical cancer risk in the single locus and diplotype models. Additionally, homozygosity for the variant allele of rs2069763 was associated with reduced risk of cervical and vulvar cancer. Although nothing is currently known regarding phenotypic consequences of this tagSNP, located in a highly conserved region of *IL2*, there is growing evidence that "silent" polymorphisms may elicit effects through subtle alterations in transcription or mRNA transport (57, 58). Furthermore, in the SeattleSNPs project, this tagSNP was in linkage disequilibrium with an intronic SNP (rs2069772) proximal (~100 bp) to the intron three-exon four junction and could feasibly alter splice factor binding. The observed reduced risk of cervical cancer associated with rs2069763 is seemingly at odds with the observation of a greater than multiplicative joint effect of rs2069763 and cigarette smoking in cervical cancer risk. These observations may be reconciled by the delicate immune balance between immunoregulation and inflammation in response to HPV infection and associated neoplastic changes. It is conceivable that the variant allele of rs2069772 is associated with reduced cervical and vulvar cancer risk via increased IL-2 activity and thus an effective regulatory T-lymphocyte response against HPV and emerging cancer cells. However, in the context of a tumor-promoting environment associated with cigarette smoking as described above, a highly effective regulatory T-lymphocyte response may be shifted towards an unregulated inflammatory response, providing a mechanism for carcinogenesis (59). The lack of a joint effect between rs2069763 and cigarette smoking in vulvar cancer risk may reflect differences between the immune responses in these tissues.

It is apparent by the *IL2* haplotypes inferred from our genotyping data that our study population exhibited a similar pattern of linkage disequilibrium to that of the SeattleSNPs population, from which our tagSNP selection was based. As each of our haplotypes was uniquely marked by a tagSNP variant allele, our haplotype models are essentially the same as log-additive single locus models. In contrast, the results from our diplotype analysis have the potential to identify joint influences of haplotypes. Carriership of one of three diplotypes together with cigarette smoking was associated with either a supra-multiplicative (TGCT/GGCC) or sub-multiplicative (TTCC/TGTC or TGCT/TGCC) joint effect in vulvar cancer risk. Due to the rarity of these diplotypes, it is possible that the observed interaction is an artifact of small numbers. Alternatively, the interaction of alleles on separate haplotypes may influence IL-2 production or function in some unknown way. The paucity of data regarding of functional consequences of these alleles makes it difficult to speculate on the biological effect of a potential interaction of alleles.

Our decision to use HPV seropositive controls for our analysis of independence and assessment of main effects ultimately influences the interpretation of the results. Immune system factors may influence HPV-associated cancer risk during (at least) three stages of disease progression: 1) upon initial HPV exposure, 2) during the establishment of a persistent HPV infection, and 3) during neoplastic progression. Seropositive controls are women who have mounted an immune response to HPV, however a proportion of these women may have developed a persistent infection while others may have encountered and cleared an infection. Furthermore, there is the possibility that women may have been exposed to an HPV infection, but did not mount an immune response and thus are not included in our control group. Our choice to include seropositive controls allows us to examine the role of *IL2* variants in the stages of disease progression beyond the initial mounting of an immune response to an HPV infection. Since the motivation for this study was to investigate a potential mechanism for

current cigarette smoking, these controls allow us to focus on the later stages of disease progression where current cigarette smoking is most likely relevant. Unfortunately, we do not have cancer-free individuals with persistent gential HPV-infection defined by HPV DNA status in our study, therefore we cannot separate our inferences regarding the joint effect of *IL2* variants and cigarette smoking, or *IL2* variants alone, on HPV persistence and tumor progression.

We chose a case-only design because it offers several advantages, including high statistical power, for exploring the role of *IL2* variation as a pathway to explain the increased risk of cervical and vulvar cancer associated with cigarette smoking. Although case-only studies are generally more powerful than case-control studies for detecting departures from multiplicative joint effects, they are still susceptible to sources of systematic error, which could lead to spurious results (60, 61). For example, selection bias could occur if a case's inclusion in this study was related jointly to her smoking status and *IL2* genotype, although this seems unlikely given that decisions to participate or provide a blood sample are made in the absence of knowledge of one's genetic makeup. Similarly, recall of information on smoking by cases is not likely to be dependent on genotype. Therefore, misclassification of smoking status will most likely be non-differential, and if present would bias the IOR towards the null. Another limitation is of the case-only study is that it can only assess effect modification on a multiplicative, as opposed to additive, scale.

Strengths of this study include the population-based recruitment of cases (and controls), attempted coverage of all common genetic variation in *IL2*, and the use of single- and multi-locus analytic methods. Furthermore, the assumption of conditional independence between the genotypes of each tagSNP and cigarette smoking in the HPV-exposed population from which the cervical and vulvar cases arise is an important foundation for this study, and we found this assumption to hold in a large sample of HPV seropositive controls.

IL-2 is central to T lymphocyte immune response, but by no means is it the only influential cytokine or immune factor to potentially modulate the effect of cigarette smoking in cervical or vulvar cancer risk. For example, cervical cancer risk is reduced among carriers of the HLA Class II DRB1*13/DBQ1*0603 alleles (62), and possibly certain polymorphisms in genes coding for interferon gamma (63) and interleukin 10 (64). The possibility that these polymorphisms, or polymorphisms of other cytokines, receptors, or immune factors, modify the association between cigarette smoking and cancer risk has yet to be explored.

Substantial progress in recent years towards development and uptake of prophylactic HPV vaccines provides hope for reducing the burden of HPV infection and associated neoplasms in the future (65). Nonetheless, there remain a large number of women that 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 regions of the world that are challenged by the high cost and distribution of a vaccine (66, 67). Identification of gene-environment interactions that contribute to cervical and vulvar cancer risk may help shed light on the biological mechanisms leading to cancer, and potentially identify women who are at increased risks of developing these malignancies.

References

1. zurHausen HT. Papillomavirus infections - A major cause of human cancers. Biochimica Et Biophysica Acta-Reviews on Cancer 1996;1288:F55-F78.

2. Madeleine MM, Daling JR, Carter JJ, et al. Cofactors with human papillomavirus in a population-based study of vulvar cancer. Journal of the National Cancer Institute 1997;89:1516-23.

3. Hildesheim A, Han CL, Brinton LA, Kurman RJ, Schiller JT. Human papillomavirus type 16 and risk of preinvasive and invasive vulvar cancer: Results from a seroepidemiological case-control study. Obstetrics and Gynecology 1997;90:748-54.

4. Castellsague X, Munoz N. Chapter 3: Cofactors in human papillomavirus carcinogenesis--role of parity, oral contraceptives, and tobacco smoking. J Natl Cancer Inst Monogr 2003;20-8.

5. Daling JR, Sherman KJ, Hislop TG, et al. Cigarette smoking and the risk of anogenital cancer. Am J Epidemiol 1992;135:180-9.

6. Bosch FX, Munoz N, de Sanjose S, et al. Risk factors for cervical cancer in Colombia and Spain. Int J Cancer 1992;52:750-8.

7. Herrero R, Brinton LA, Reeves WC, et al. Invasive cervical cancer and smoking in Latin America. J Natl Cancer Inst 1989;81:205-11.

8. Szarewski A, Jarvis MJ, Sasieni P, et al. Effect of smoking cessation on cervical lesion size. Lancet 1996;347:941-43.

9. Vineis P, Alavanja M, Buffler P, et al. Tobacco and cancer: recent epidemiological evidence. J Natl Cancer Inst 2004;96:99-106.

10. Frisch M, Biggar IJ, Goedert JJ. Human papillomavirus-associated cancers in patients with human immunodeficiency virus infection and acquired immunodeficiency syndrome. Journal of the National Cancer Institute 2000;92:1500-10.

11. Moscicki AB, Ellenberg JH, Vermund SH, et al. Prevalence of and risks for cervical human papillomavirus infection and squamous intraepithelial lesions in adolescent girls - Impact of infection with human immunodeficiency virus. Archives of Pediatrics & Adolescent Medicine 2000;154:127-34.

12. Halpert R, Fruchter RG, Sedlis A, et al. Human papillomavirus and lower genital neoplasia in renal transplant patients. Obstet Gynecol 1986;68:251-8.

13. Nakagawa M, Stites DP, Farhat S, et al. Cytotoxic T lymphocyte responses to E6 and E7 proteins of human papillomavirus type 16: relationship to cervical intraepithelial neoplasia. J Infect Dis 1997;175:927-31.

14. Eiben GL, Velders MP, Kast WM. The cell-mediated immune response to human papillomavirus-induced cervical cancer: implications for immunotherapy. Adv Cancer Res. 2002;86:113-48.

15. Clerici M, Merola M, Ferrario E, et al. Cytokine production patterns in cervical intraepithelial neoplasia: association with human papillomavirus infection. J Natl Cancer Inst 1997;89:245-50.

16. Stanley MA. Immunobiology of papillomavirus infections. J Reprod Immunol 2001;52:45-59.

17. Curfs JH, Meis JF, Hoogkamp-Korstanje JA. A primer on cytokines: sources, receptors, effects, and inducers. Clin Microbiol Rev 1997;10:742-80.

18. de Gruijl TD, Bontkes HJ, Walboomers JM, et al. Differential T helper cell responses to human papillomavirus type 16 E7 related to viral clearance or persistence in patients with cervical neoplasia: a longitudinal study. Cancer Res 1998;58:1700-06.

19. Al-Saleh W, Giannini SL, Jacobs N, et al. Correlation of T-helper secretory differentiation and types of antigen-presenting cells in squamous intraepithelial lesions of the uterine cervix. J Pathol. 1998;184:283-90.

20. Li Q, Aubrey MT, Christian T, Freed BM. Differential inhibition of DNA synthesis in human T cells by the cigarette tar components hydroquinone and catechol. Fundam Appl Toxicol 1997;38:158-65.

21. Geiselhart LA, Christian T, Minnear F, Freed BM. The cigarette tar component p-benzoquinone blocks T-lymphocyte activation by inhibiting interleukin-2 production, but not CD25, ICAM-1, or LFA-1 expression. Toxicol Appl Pharmacol 1997;143:30-6.

22. McCue JM, Link KL, Eaton SS, Freed BM. Exposure to cigarette tar inhibits ribonucleotide reductase and blocks lymphocyte proliferation. J Immunol 2000;165:6771-5.

23. Madretsma GS, Donze GJ, van Dijk AP, et al. Nicotine inhibits the in vitro production of interleukin 2 and tumour necrosis factor-alpha by human mononuclear cells. Immunopharmacology 1996;35:47-51.

24. Ouyang Y, Virasch N, Hao P, et al. Suppression of human IL-1beta, IL-2, IFN-gamma, and TNF-alpha production by cigarette smoke extracts. J Allergy Clin Immunol 2000;106:280-7.

25. Hoffmann SC, Stanley EM, Darrin Cox E, et al. Association of cytokine polymorphic inheritance and in vitro cytokine production in anti-CD3/CD28-stimulated peripheral blood lymphocytes. Transplantation 2001;72:1444-50.

26. Schiffman MH, Castle P. Epidemiologic studies of a necessary causal risk factor: human papillomavirus infection and cervical neoplasia. J Natl Cancer Inst 2003;95:E2.

27. Sellors JW, Karwalajtys TL, Kaczorowski JA, et al. Prevalence of infection with carcinogenic human papillomavirus among older women. CMAJ 2002;167:871-3.

28. Khoury MJ, Flanders WD. Nontraditional epidemiologic approaches in the analysis of gene-environment interaction: case-control studies with no controls! Am J Epidemiol 1996;144:207-13.

29. Yang Q, Khoury MJ, Flanders WD. Sample size requirements in case-only designs to detect gene-environment interaction. Am J Epidemiol 1997;146:713-20.

30. Daling JR, Madeleine MM, McKnight B, et al. The relationship of human papillomavirus-related cervical tumors to cigarette smoking, oral contraceptive use, and prior herpes simplex virus type 2 infection. Cancer Epidemiol Biomarkers Prev 1996;5:541-8.

31. Hankey BF, Ries LA, Edwards BK. The surveillance, epidemiology, and end results program: a national resource. Cancer Epidemiol Biomarkers Prev 1999;8:1117-21.

32. Waksberg J. Sampling methods for random digit dialing. J Am Stat Assoc 1978;73:40-46.

33. Hartge P, Brinton LA, Rosenthal JF, et al. Random digit dialing in selecting a population-based control group. Am J Epidemiol 1984;120:825-33.

34. Carter JJ, Madeleine MM, Shera K, et al. Human papillomavirus 16 and 18 L1 serology compared across anogenital cancer sites. Cancer Res 2001;61:1934-40.

35. Madeleine MM, Anttila T, Schwartz SM, et al. Risk of cervical cancer associated with Chlamydia trachomatis antibodies by histology, HPV type and HPV cofactors. International Journal of Cancer 2007;120:650-55.

36. Seattle SNPs. NHLBI Program for Genomic Applications, UW-FHCRC, Seattle, WA (URL: http://pga.gs.washington.edu) [August, 2003].

37. Carlson CS, Eberle MA, Rieder MJ, et al. Selecting a maximally informative set of single-nucleotide polymorphisms for association analyses using linkage disequilibrium. Am J Hum Genet 2004;74:106-20.

38. Garcia-Closas M, Egan KM, Abruzzo J, et al. Collection of genomic DNA from adults in epidemiological studies by buccal cytobrush and mouthwash. Cancer Epidemiol Biomarkers Prev 2001;10:687-96.

39. Umbach DM, Weinberg CR. Designing and analysing case-control studies to exploit independence of genotype and exposure. Stat Med 1997;16:1731-43.

40. Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. Am J Hum Genet 2001;68:978-89.

41. French B, Lumley T, Monks SA, et al. Simple estimates of haplotype relative risks in case-control data. Genetic Epidemiology 2006;30:485-94.

42. Sasson IM, Haley NJ, Hoffmann D, et al. Cigarette smoking and neoplasia of the uterine cervix: smoke constituents in cervical mucus. N Engl J Med 1985;312:315-6.

43. Poppe WA, Ide PS, Drijkoningen MP, Lauweryns JM, Van Assche FA. Tobacco smoking impairs the local immunosurveillance in the uterine cervix. An immunohistochemical study. Gynecol Obstet Invest 1995;39:34-8.

44. Barton SE, Maddox PH, Jenkins D, et al. Effect of cigarette smoking on cervical epithelial immunity: a mechanism for neoplastic change? Lancet 1988;2:652-4.

45. Crowley-Nowick PA, Ellenberg JH, Vermund SH, et al. Cytokine profile in genital tract secretions from female adolescents: impact of human immunodeficiency virus, human papillomavirus, and other sexually transmitted pathogens. J Infect Dis 2000;181:939-45.

46. Kuhn RM, Karolchik D, Zweig AS, et al. The UCSC Genome Browser Database: Update 2007. Nucleic Acids Research 2007;35:D668-D73.

47. Siepel A, Bejerano G, Pedersen JS, et al. Evolutionarily conserved elements in vertebrate, insect, worm, and yeast genomes. Genome Research 2005;15:1034-50.

48. Carlson JA, Ambros R, Malfetano J, et al. Vulvar lichen sclerosus and squamous cell carcinoma: A cohort, case control, and investigational study with historical perspective; Implications for chronic inflammation and sclerosis in the development of neoplasia. Human Pathology 1998;29:932-48.

49. MacMillan ML, Radloff GA, Kiffmeyer WR, et al. High-producer interleukin-2 genotype increases risk for acute graft-versus-host disease after unrelated donor bone marrow transplantation. Transplantation 2003;76:1758-62.

50. Ahn KS, Aggarwal BB. Transcription factor NF-kappa B - A sensor for smoke and stress signals. Natural Products and Molecular Therapy, Annals of the New York Academy of Sciences 1056, pp. 218-33, 2005.

51. Zeidler R, Albermann K, Lang S. Nicotine and apoptosis. Apoptosis 2007;12:1927-43.

52. Bontkes HJ, de Gruijl TD, van den Muysenberg AJC, et al. Human papillomavirus type 16 E6/E7-specific cytotoxic T lymphocytes in women with cervical neoplasia. International Journal of Cancer 2000;88:92-98.

53. Todd RW, Roberts S, Mann CH, et al. Human papillomavirus (HPV) type 16-specific CD8+ T cell responses in women with high grade vulvar intraepithelial neoplasia. Int J Cancer 2004;108:857-62.

54. Davidson EJ, Boswell CM, Sehr P, et al. Immunological and clinical responses in women with vulval intraepithelial neoplasia vaccinated with a vaccinia virus encoding human papillomavirus 16/18 oncoproteins. Cancer Res 2003;63:6032-41.

55. Thorisson GA, Smith AV, Krishnan L, Stein LD. The International HapMap Project Web site. Genome Research 2005;1591-93.

56. Schumacher JM, Lee K, Edelhoff S, Braun RE. Distribution of Tenr, an Rna-Binding Protein, in a Lattice-Like Network within the Spermatid Nucleus in the Mouse. Biology of Reproduction 1995;52:1274-83.

57. Kimchi-Sarfaty C, Oh JM, Kim IW, et al. A "silent" polymorphism in the MDR1 gene changes substrate specificity. Science 2007;315:525-28.

58. Chamary JV, Parmley JL, Hurst LD. Hearing silence: non-neutral evolution at synonymous sites in mammals. Nature Reviews Genetics 2006;7:98-108.

59. Coussens LM, Werb Z. Inflammation and cancer. Nature 2002;420:860-7.

60. Garcia-Closas M, Rothman N, Lubin J. Misclassification in case-control studies of gene-environment interactions: assessment of bias and sample size. Cancer Epidemiol Biomarkers Prev 1999;8:1043-50.

61. Rothman N, Garcia-Closas M, Stewart WT, Lubin J. The impact of misclassification in case-control studies of gene-environment interactions. IARC Sci Publ 1999;89-96.

62. Hildesheim A, Wang SS. Host and viral genetics and risk of cervical cancer: a review. Virus Res 2002;89:229-40.

63. Lai HC, Chang CC, Lin YW, et al. Genetic polymorphism of the interferon-gamma gene in cervical carcinogenesis. Int J Cancer 2005;113:712-18.

64. Stanczuk GA, Sibanda EN, Perrey C, et al. Cancer of the uterine cervix may be significantly associated with a gene polymorphism coding for increased IL-10 production. Int J Cancer 2001;94:792-4.

65. Stanley MA. Human papillomavirus vaccines. Reviews in Medical Virology 2006;16:139-49.

66. Hildesheim A, Herrero R, Wacholder S, et al. Effect of human papillomavirus 16/18 L1 viruslike particle vaccine among young women with preexisting infection - A randomized trial. Jama-Journal of the American Medical Association 2007;298:743-53.

67. Schiller JT, Davies P. Science and society - Delivering on the promise: HPV vaccines and cervical cancer. Nature Reviews Microbiology 2004;2:343-47.

671

Table 1. Selected characteristics of cervical and vulvar squamous cell carcinoma cases

	Cervical cancer cases (N=399)	Vulvar cancer cases (N=486)
Mean age at diagnosis (years)	43.1	47.4
Tumor stage at diagnosis by FIGO staging (%)		
Vulvar		
0		88.6
1+		11.4
Cervix		
<2b	82.9	
≥2b	17.1	
HPV DNA Testing (%)		
Not tested	36.6	28.6
Tested	63.4	71.4
Positive Result (high risk types)*	83.4	82.3
Negative Result (high risk types)*	12.5	10.0
Undetermined*	4.1	7.7
Education (%)		
High school or less	37.9	37.9
Less than 4 years of college or technical school	41.3	42.1
4 years of college or more	20.8	20.0
Cigarette Smoking (%)		
Never	38.4	20.2
Former	26.9	22.6
Current	34.7	57.2
Number of lifetime sexual partners (%)		
1	9.6	7.6
2 to 4	30.5	21.5
5 to 14	44.1	43.8
≥15	15.9	27.1
Number of births (%)		
0	18.1	29.6
1	16.3	19.3
2	30.2	25.5
≥3	35.4	25.5
Duration of oral contraceptive use (%)		
Never or less than 6 months	31.4	29.6

6 to 59 months	31.4	32.3
≥5 years	37.2	38.1
First degree relative with anogenital cancer (%)		
Yes	3.7	8.0
No	96.3	92.0

* Represents the percentage of tested individuals

Table 2. *IL2* tagSNP characteristics, smoking prevalence, and results from tests of independence of *IL2* tagSNPs and cigarette smoking in controls

TagSNP*	Location†	Gene feature‡	Alleles (common/variant)	Variant allele frequency	Smoking prevalence§	Cervix controls		Vulvar control
						OR (95% CI)‖	*p* value**	OR (95% CI)
rs2069762	495	5'flanking	T/G	0.23	23%	1.19 (0.69-2.06)	0.68	1.30 (0.82-2.04)
rs2069763	993	Exon 1	G/T	0.38	23%	1.11 (0.66-1.85)	0.48	1.08 (0.69-1.67)
rs2069777	2038	Intron 1	C/T	0.07	21%	1.23 (0.45-3.37)	0.69	0.90 (0.34-2.33)
rs2069778	2340	Intron 1	C/T	0.18	18%	0.72 (0.35-1.46)	0.54	0.60 (0.31-1.11)

*rs number refers to the National Center for Bioinformatics (NCBI) dbSNP build 127 reference sequence number.

†Locations are with respect to the first nucleotide position in the NCBI GenBank entry: accession number AF359939.

‡Location of tagSNP within gene: 5' flanking is upstream of the first exon of the gene, exon is in the coding region of the gene, intron is between codin of the gene.

§Prevalence of current cigarette smoking among all controls (n=236) who carried at least one copy of the variant allele for each tagSNP

‖OR, odds ratio; CI, confidence interval. The ORs are the exponentiated joint effect parameters for cigarette smoking and tagSNP genotype among cont from the full model (39) assuming a log-additive genetic model. These can be interpreted as the association between *IL2* tagSNP genotypes and cigarett smoking among controls.

**Likelihood ratio test *p* value (39)

Table 3. Interaction odds ratios between *IL2* genotypes and cigarette smoking on cervical and vulvar cancer risk

TagSNP*	Genotype	Cervical cancer cases			Vulvar cancer cases		
		Genotype frequency		IOR (95% CI)†	Genotype frequency		IOR (95% CI)
		Smokers	Non-smokers		Smokers	Non-smokers	
rs2069762	TT	0.54	0.51	1.00	0.42	0.56	1.00
	TG	0.37	0.42	0.85 (0.55-1.32)	0.50	0.38	1.69 (1.15-2.47)
	GG	0.09	0.07	1.29 (0.60-2.80)	0.08	0.06	1.59 (0.76-3.32)
	TG or GG vs. TT‡			0.91 (0.60-1.38)			1.67 (1.16-2.41)
	GG vs. TT or TG§			1.39 (0.66-2.93)			1.24 (0.61-2.54)
rs2069763	GG	0.44	0.42	1.00	0.44	0.43	1.00
	GT	0.39	0.48	0.79 (0.50-1.25)	0.48	0.49	0.98 (0.67-1.44)
	TT	0.17	0.10	1.66 (0.86-3.22)	0.08	0.08	0.98 (0.48-1.97)
	GT or TT vs. GG‡			0.94 (0.61-1.43)			0.98 (0.68-1.42)
	TT vs. GG or GT§			1.87 (1.00-3.48)			0.99 (0.50-1.94)
rs2069777	CC	0.86	0.85	1.00	0.86	0.80	1.00
	CT	0.14	0.15	0.96 (0.53-1.74)	0.14	0.19	0.65 (0.39-1.06)
	TT			—	0.01	0.01	0.72 (0.04-11.6)
	CT or TT vs. CC‡			—			0.65 (0.40-1.06)
	TT vs. CC or CT§			—			0.77 (0.05-12.40)
rs2069778	CC	0.74	0.68	1.00	0.66	0.69	1.00
	CT	0.22	0.28	0.70 (0.43-1.16)	0.31	0.29	1.17 (0.78-1.75)
	TT	0.04	0.04	1.15 (0.40-3.33)	0.03	0.02	1.42 (0.41-4.95)
	CT or TT vs. CC‡			0.75 (0.47-1.20)			1.18 (0.80-1.80)
	TT vs. CC or CT§			1.26 (0.44-3.62)			1.35 (0.39-4.69)

* rs number refers to the National Center for Bioinformatics (NCBI) dbSNP build 127 reference sequence number.

†IOR, interaction odds ratio; CI, confidence interval.

‡Dominant genetic model.

§Recessive genetic model.

Table 4. Interaction odds ratios between *IL2* haplotypes and cigarette smoking on cervical and vulvar cancer risk based on a log-additive model

Haplotype*	Cervical cancer cases			Vulvar cancer cases		
	Haplotype frequency		IOR (95% CI)†	Haplotype frequency		IOR (95% CI)
	Smokers	Non-smokers		Smokers	Non-smokers	
TTCC	0.37	0.33	1.00	0.32	0.32	1.00
GGCC	0.28	0.28	0.93 (0.63-1.36)	0.32	0.25	1.34 (0.94-1.92)
TGCT	0.15	0.18	0.81 (0.52-1.26)	0.18	0.16	1.16 (0.79-1.71)
TGCC	0.13	0.14	0.89 (0.57-1.41)	0.11	0.16	0.71 (0.47-1.07)
TGTC	0.07	0.07	0.90 (0.49-1.65)	0.07	0.10	0.65 (0.39-1.07)

*Alleles in each haplotype are listed from 5' to 3' (rs2069762, rs2069763, rs2069777, and rs2069778). Variant alleles are underlined.

†IOR, interaction odds ratio; CI, confidence interval. Calculated assuming a log-additive genetic model.

Table 5. Interaction odds ratios between *IL2* diplotypes and cigarette smoking on cervical and vulvar cancer risk

Diplotype*	Cervical cancer cases			Vulvar cancer cases		
	Diplotype frequency		IOR (95% CI)†	Diplotype frequency		IOR (95% CI)
	Smokers	Non-smokers		Smokers	Non-smokers	
T<u>T</u>CC / <u>G</u>GCC	0.18	0.22	1.00	0.25	0.20	1.00
T<u>T</u>CC / T<u>T</u>CC	0.17	0.10	2.08 (1.01-4.30)	0.08	0.08	0.85 (0.41-1.78)
T<u>T</u>CC / TGC<u>T</u>	0.08	0.12	0.83 (0.37-1.87)	0.11	0.11	0.82 (0.43-1.56)
T<u>T</u>CC / TGCC	0.11	0.08	1.69 (0.76-3.79)	0.07	0.08	0.74 (0.36-1.51)
TGC<u>T</u> / <u>G</u>GCC	0.08	0.09	1.09 (0.47-2.53)	0.13	0.05	2.09 (0.98-4.46)
<u>G</u>GCC / <u>G</u>GCC	0.09	0.07	1.68 (0.72-3.95)	0.07	0.06	0.97 (0.44-2.13)
TGCC / <u>G</u>GCC	0.05	0.06	1.05 (0.41-2.72)	0.05	0.08	0.55 (0.26-1.17)
T<u>T</u>CC / TG<u>T</u>C	0.03	0.05	0.72 (0.22-2.34)	0.04	0.09	0.37 (0.16-0.85)
TG<u>T</u>C / <u>G</u>GCC	0.06	0.04	1.70 (0.63-4.58)	0.05	0.03	1.25 (0.47-3.33)
TGC<u>T</u> / TGCC	0.03	0.06	0.65 (0.20-2.08)	0.03	0.08	0.37 (0.15-0.87)
TGC<u>T</u> / TGC<u>T</u>	0.04	0.03	1.66 (0.54-5.11)	0.02	0.02	0.95 (0.26-3.47)
TGC<u>T</u> / TG<u>T</u>C	0.03	0.02	2.28 (0.55-9.37)	0.03	0.04	0.53 (0.19-1.45)
TGCC / TGCC	0.02	0.02	1.65 (0.38-7.19)	0.02	0.02	0.72 (0.20-2.55)
TGCC / TG<u>T</u>C	0.02	0.04	0.77 (0.20-3.04)	0.01	0.03	0.35 (0.10-1.22)

*The two haplotypes carried on each chromosome are separated by the "/". Alleles in each haplotype are listed from 5' to 3' (rs2069762, rs2069763, rs2069777, and rs2069778). Variant alleles are underlined.

†IOR, interaction odds ratio; CI, confidence interval.

Table 6. Main effect of each tagSNP on cervical and vulvar cancer risk

TagSNP*	Genotype	OR (95% CI)†	
		Cervical cancer	Vulvar cancer
rs2069762	TT	1.00	1.00
	TG	1.14 (0.79-1.66)	1.28 (0.92-1.78)
	GG	1.15 (0.57-2.29)	0.84 (0.47-1.50)
rs2069763	GG	1.00	1.00
	GT	0.78 (0.53-1.15)	0.97 (0.69-1.37)
	TT	0.60 (0.35-1.04)	0.45 (0.27-0.76)
rs2069777	CC	1.00	1.00
	CT	1.12 (0.66-1.89)	1.33 (0.84-2.11)
rs2069778	CC	1.00	1.00
	CT	0.87 (0.58-1.30)	1.03 (0.73-1.47)
	TT	1.31 (0.47-3.69)	0.67 (0.27-1.71)

*rs number refers to the National Center for Bioinformatics (NCBI) dbSNP build 127 reference sequence number.
†OR, odds ratio; CI, confidence interval. All controls were seropositive for HPV16 or HPV18 L1 antibodies.

Supplementary Table 1. Assay primer and probe sequences for *IL2* tagSNPs*

TagSNP†	rs2069763	rs2069777	rs2069778
Forward primer sequence	5'-TGCACCTACTTCAAGTTCTACAAAGAA-3'	5'-CATCCAAGCTCCTAGGCTACATTAG-3'	5'-GCTGTTTTCTGAAGAAAATTTCTCCACAT-3'
Reverse primer sequence	5'-AAAGGAAATATACTTACATTAATTCCATTCAAAATCATCTG-3'	5'-TGGCACCAGATTTTGTTCATTCTCT-3'	5'-GCGCTTTCAATTCACCACTACAA-3'
Probe sequence 1‡	5'-ATCCAGCAGTAAATG-3'	5'-ACTGGCACAGCTACTA-3'	5'-TTCTACAAATTCGGGTTTAA-3'
Probe sequence 2§	5'-TAAATCCAGAAGTAAATG-3'	5'-ACTGGCACAACTACTA-3'	5'-ATTCTACAAATTCAGGTTTAA-3'
Orientation	Forward	Forward	Forward

*The rs2069762 was genotyped using Applied Biosystems Pre-Designed Taqman® genotyping assay for which the probes and primer sequences are proprietary information.

†Rs number refers to the National Center for Bioinformatics (NCBI) dbSNP build 127 reference sequence number.

‡Probe sequences were labeled with a 5' reporter VIC dye and 3' minor groove binding non-fluorescent quencher.

§Probe sequences were labeled with a 5' reporter FAM dye and 3' minor groove binding non-fluorescent quencher.