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## A population-based case-control study of genetic variation in cytokine genes associated with risk of cervical and vulvar cancers

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### Abstract

**Objective**—Persistent infection with oncogenic human papillomavirus (HPV) is known to be the necessary cause of cervical cancer and a majority of vulvar cancers. Persistent HPV infections must evade host immune responses, including cytokines released by activated T-helper (Th) cells. In this study, we investigated the risk of cervical and vulvar cancer associated with common genetic variations in 560 tagging single-nucleotide polymorphisms (SNPs) in candidate cytokine genes.

**Methods**—The study included 399 invasive squamous cell carcinomas (SCC) and 502 in situ or invasive adenocarcinomas (AC) of the cervix; 357 in situ or invasive vulvar SCC; and 1,109 controls from the Seattle-area case-control studies of HPV-related cancers. Logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (CI) using a log additive model, with adjustment for multiple testing.

**Results**—Statistically significant risks were observed for HPV16-containing SCC of the cervix with the variant allele rs879576 in *IL17RA* and rs2229094 in *TNF* [OR, 95% CI and multiple-testing corrected p: 1.91(1.30-2.79), p= 0.018 and 0.61 (0.45-0.83), p= 0.02, respectively]. We also observed significantly increased risk of HPV-positive vulvar cancers associated with variant alleles in *CSF2* (rs25882 and rs27438, 26-28% increased risk) and *IL-12B* (rs2569254 and rs3181225, 40-41% increased risk) genes.

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Conflict of Interest Statement

The authors do not have any potential conflicts of interest to declare.

**Conclusions**—We found that variation in several Th-cytokine genes are significantly associated with cervical and vulvar cancer risk. The strong association between these HPV-related cancers and common variation in cytokine genes in the Th1 and Th17 pathways may be important for development of new therapies.

### Keywords

Cervical cancer; Vulvar cancer; T-helper 1 pathways; T-helper 2 pathway; T-helper 17 pathway; genetic variation

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### Introduction

Although the burden of carcinoma of the cervix has decreased considerably in countries with wide-spread screening, it remains the third most commonly occurring cancer and fourth most common cause of cancer deaths among women worldwide.[1] Persistent infection with oncogenic human papillomavirus (HPV) has been established as the necessary cause for the development of cervical cancer and a large proportion of HPV-related cancers at other anogenital sites, including vulvar cancer.[2] In contrast with the overall decreased incidence of cervical cancer, there has been a steady rise in the incidence of cervical adenocarcinoma (AC) and squamous cell carcinoma (SCC) of the vulva.[3, 4] HPV infections alone are not sufficient for neoplastic progression to these cancers, as transient HPV infections are extremely common in the general population and relatively few women infected with HPV progress to cancer.[5, 6] The mechanism of clearance of transient infections is complex: it involves HPV antigen presentation to CD4 and CD8 T cells, leading to activated T cells that proliferate into armed effector T cells. [7] In the small group of women who progress from HPV persistent infection to HPV-related cancer, HPV evades detection mainly through activation of the adaptive immune system.[8]

Evidence of the role of adaptive immunity comes from studies that assess T-cells generated in response to HPV infection and lesion development.[9, 10] Those studies suggest that T helper (Th) cells are necessary for production of HPV-specific antibody response and in aiding the development of cytotoxic T lymphocytes. Th responses that result in predominantly Th1-type cytokines favor HPV clearance (Figure 1), whereas activation and terminal differentiation of Th2 cells leads to higher levels of circulating pro-inflammatory cytokines and fewer HPV-specific T-cell responses.[9, 10] Th17 cells are induced by TGFB and produce IL-17-related pro-inflammatory cytokines that act to suppress HPV response. [11]

Further evidence of the role of immunity in HPV-related cancers comes from natural history studies, which demonstrated that most young women clear the virus within 1-2 years of infection.[12] In contrast, however, a high burden of HPV-related cancers has been observed in several immunosuppressed populations, suggesting that cancer is more likely to develop among individuals with compromised adaptive immune responses.[13] HPV-associated malignancies occur in excess among patients with HIV and AIDS, probably due to reduced HPV clearance among HIV/AIDS patients with low CD4 T-helper cell counts.[13] Incidence of HPV-related cancers is also greatly increased following solid organ

transplantation, and these risks increase with length of time on immune suppression.[14] In a rare genetic disease, individuals with WHIM syndrome (OMIM #193670) have mutations in a chemokine receptor, CXCR4, which leads to impaired chemotaxis of leukocytes and inability to clear HPV infections.[15] These studies suggest that the adaptive immune responses are critical to HPV progression to cancer.

In prior publications involving single genes (or a group of genes) within immune response pathways, our research group has found several statistically significant associations with cervical cancer subtypes.[16-19] In this paper, we conducted a comprehensive analysis of genes within the three primary immune response pathways: Th1, Th2 and Th17, and their association with SCC of the cervix, AC of the cervix and vulvar cancers. We focused on subsets, such as HPV16 positive squamous cell and adenocarcinoma of the cervix to determine if HPV16, the most prevalent cause of cervical cancer, was associated with the candidate genes across histologic types. We investigated whether genetic polymorphisms in cytokine genes involved in adaptive immunity, specifically genes important in the Th1, Th2 and Th17 immune response pathway, are associated with cervical and vulvar cancer risk in a population-based case-control study in western Washington state, in the northwest US.

## Methods

### Study population and data collection

The present analysis utilized data from participants within a large population-based case-control study aimed at understanding the role of host and environmental factors in anogenital cancers. The recruitment procedures, response rates, and details of sample collection have been described elsewhere,[20-22] and are briefly summarized here. Eligible cases were women 18-74 years of age diagnosed between 1986 and 2004 with incident cervical or vulvar cancers (invasive SCC of the cervix, ICDO codes 8010-8081; invasive or *in situ* AC, ICDO codes 8140-8480; and invasive or *in situ* SCC of the vulva, ICDO codes 8010, 8070-8077, 8081) while residing in Western Washington state. Case women were identified through the Cancer Surveillance System, a population-based cancer registry that is part of the NCI Surveillance Epidemiology and End Results (SEER) program.[23] Controls were residents of the same region as the cases and were selected through random digit telephone dialing. The controls were frequency matched to the distribution of age at diagnosis for the case groups, in 5-year age intervals. The current report is restricted to white females (91.3%) that had reported at least one sexual partner (99.3%). This study was approved by the Institutional Review Board at the Fred Hutchinson Cancer Research Center, Seattle.

### Laboratory Methods

Participants provided peripheral blood samples at the time of interview, from which DNA was extracted. A total of 667 tagging single nucleotide polymorphisms (tagSNPs) were selected from 52 genes in the Th1, Th2 and Th17 families of cytokines (pairwise  $r^2 > 0.80$  used to delineate highly correlated SNPs), with MAF  $> 5\%$  used for each gene. We used the Snagger algorithm applied to the CEU HapMap population and the Seattle SNPs Variation Discovery Resource to select tagging SNPs.[24] For most groups of correlated SNPs, one

tagSNP was selected, however, wherever necessary, we selected multiple SNPs per group to guard against missed coverage due to genotyping failure. For each gene or gene region, the chromosomal location for choosing tagSNPs extended 4kb beyond the 3' and the 5' ends to capture variation that may influence gene function.

Genotyping and quality control measures were followed as reported previously.[17, 19] Briefly, Illumina Golden Gate platform (Illumina Inc., San Diego, CA) was used to type the 667 SNPs. Although two SNPs had a Hardy-Weinberg equilibrium (HWE) p-value below 0.05, none had a HWE p-value below 0.00004 (0.05/1288, total number of passing SNPs assayed on the platform, including those not in this study) and hence none of the SNPs were excluded based on HWE. A subset of the 94 failed SNPs on the Illumina platform was assayed using the KBioscience allele-specific PCR system (KBioscience, Hoddesdon, UK) or TaqMan assays (Life Technologies, Grand Island, NY), leading to a total of 560 SNPs, across all three platforms, included for analyses. Data quality control was achieved through visual inspection of the signal intensity plots by two independent readers. Any SNPs with call frequency below 0.9 were excluded. Genotype data were also evaluated for completeness. There was 1 SNP on the Illumina platform and 8 SNPs on the Taqman platform with more than 5% missing data. Two out of these nine SNPs (*IL-10RA* rs2229113 and *IL-12B* rs2569254) were significantly associated with AC of the cervix, as noted in the results.

Our group has previously published on the risk of cervical and vulvar cancers and genetic variation in some of the genes included in the present analyses (*IL-2*, *IL-10*, *IL-12*, *TNF* and their associated receptors in relation to risk of SCC and AC of cervix as well as SCC of vulva; *CSF2*, *IL-3*, *IL4*, *IL13* and associated receptors in relation to risk of SCC of cervix). [16-19] Associations described in prior publications from this dataset are clearly marked as previously published in the tables. These data are presented again for comparison to results restricted to HPV16-positive subgroups, and the new analyses are the focus of this report. New to this manuscript are the following genes and associated receptors (*IFNG*, *IFNG-R1*, *IFNG-R2*, *IL17A*, *IL17B*, *IL17F*, *IL17RA*, *IL17RB*, *IL18*, *IL18BP*, *IL18R1*, *IL18RAP*, *IL1A*, *IL1B*, *IL1R1-IL1RL2*, *IL1R2*, *IL1RL1*, *IL1RN*, *IL23A*, *IL23R*, *IL25[IL17E]*, *IL27*, *IL27RA*, *IL2RA*, *IL6*, *IL6R*, *TGFB2*, *TGFB1*, *TGFB2*, *TGFB3*, *TGFBR1*, *TGFBR2*).

Presence and type of HPV DNA in paraffin-embedded tumor tissue from biopsies or surgical specimens was previously assessed for cervical and vulvar tumors. We used polymerase chain reaction (PCR) methods to detect HPV DNA in tumors as previously described.[20, 25] Briefly, PCR was performed using MY09/MY11 L1 consensus primers and HPV 16 and HPV18 E6 primers. The identity of the PCR products was confirmed by Southern hybridization, and the L1 consensus products were typed by restriction fragment analysis. Additionally, coamplification of a 536-bp or a 268-bp fragment of the human  $\beta$ -globin gene was conducted to assess specimen adequacy for presence of human DNA. There were HPV DNA PCR results available for 1006 (80%) of the 1258 case subjects included in this analysis.

## Statistical analysis

The main analyses comprised of 901 cervical cancers (44% SCC (n=399) and 56% AC (n=502)), 357 cases of HPV-positive vulvar SCC, and each of these case groups was compared with 1109 population-based controls. In secondary analyses, we examined subgroups that had HPV16 positive tumors. We found 158 (158/399, 39.6%) of the cervical SCC tumors had HPV16 in their tumor tissue. A lesser proportion of AC (136/502, 27.1%) were observed to contain HPV16. 85.33% of vulvar cancers were HPV16 positive in our data, hence we looked at all HPV-positive cancers for vulvar SCC. The demographic characteristics between these various subgroups were tested for statistically significant differences using ANOVA or chi-square tests, as appropriate.

Unconditional logistic regression analysis was used to estimate per-allele odds ratios (OR) and 95% confidence intervals (95% CI) for the association of each SNP with all the case groups using a log additive model. Age-adjusted risk estimates were obtained. For SNPs with very low counts of the homozygous variant (less than 5) in either the case or control group, genotypes were modeled assuming a dominant model. *P*-values less than 0.05 were considered to be significant. The Holm step-down procedure was applied to correct for multiple comparisons on a per-gene basis for each cancer phenotype, and the corrected values reported. All statistical analyses were performed using Stata version 13 (StataCorp, College Station, TX).

## Results

Selected demographic characteristics of study cases and controls are presented in Table 1. The mean ages of women presenting with different sub-types of cervical cancers were different ( $p_{ANOVA} < 0.001$ ); women presenting with AC of cervix were relatively younger (38.4 years) than those presenting with SCC of cervix (42.6 years). Vulvar carcinoma cases were older (47.2 years) compared to controls (45.6 years) and cervical cancer cases. Compared with control subjects, women with SCC of the cervix and vulva were less educated ( $p_{chi-sq} < 0.001$ ) and more likely to have more than 5 lifetime sexual partners ( $p_{chi-sq} < 0.001$ ). Women with vulvar carcinoma were more likely to be current smokers (60.8%) than both controls (20.7%) and cervical cancer cases (27.3%) ( $p_{chi-sq} < 0.001$ ). Smoking habits and educational attainment for women with AC of the cervix was more similar to controls than to women with SCC of the cervix.

Of the 667 genotyped SNPs, 94 failed to amplify and 13 were found to be monomorphic and were excluded from further analysis. The results for the remaining 560 SNPs are described by pathway. Of the 318 SNPs from the Th1 pathway genes and their receptors that were evaluated, 64 SNPs were significantly associated with either cervical or HPV-positive vulvar cancers or both (Table 2) before correction for multiple testing, and 13 SNPs within *CSF2*, *IL-12B* and *TNF* genes remained significant after correction for multiple testing. Results not previously published include a missense C/T variant in *CSF2* gene (rs25882) that displayed a significant association with HPV-positive vulvar cancers (OR 1.28, 95% CI 1.04-1.57; corrected  $p = 0.03$ ), and an A/G variant in the intergenic SNP rs27438 for *CSF2* gene (OR 1.26, 95% CI 1.03-1.54; corrected  $p = 0.02$ ). A 40% increased risk of HPV-positive vulvar cancers was also observed for minor allele-containing genotypes of two intronic

SNPs in *IL-12B* gene: rs2569254 (OR 1.41, 95% CI 1.13-1.76; corrected p= 0.03) and rs3181225 (OR 1.40, 95% CI 1.13-1.73; corrected p=0.02). Several tagSNPs in the *TNF*-gene were also found to be associated with cervical and vulvar cancer risk even after multiple test correction, three of which have not been previously reported by our group. The minor allele (G) of rs2229094 missense variant in the *TNF*-gene was significantly associated with a decreased risk of HPV16 positive cervical cancer (OR 0.61, 95% CI 0.45-0.83; corrected p= 0.02). We also found a 23% decreased risk for cervical adenocarcinomas associated with the minor allele (A) of rs915654 in the *TNF*-gene (OR 0.77, 95% CI 0.65-0.91; corrected p = 0.02). Finally, a 65% increased risk of HPV-positive vulvar cancer was seen with the minor allele (A) of rs769178 in the *TNF* region (OR 1.65, 95% CI 1.20-2.26; corrected p= 0.01).

We tested 245 SNPs in Th2 genes for their associations with the five case groups for cervical and vulvar cancers. There were 22 significant associations between single SNPs and the five case groups. Except for the significant associations with *IL-10* and its associated receptors that we previously reported, none had been previously looked at by our group. However, none of these associations remained statistically significant after correction for multiple testing (Table 3).

For Th17, we tested 69 SNPs in the *IL-17*, *IL-23* and *IL-25* gene families and their receptors to evaluate the association between variation in these gene families and risk for cervical and vulvar cancers. We observed that 14 SNPs showed a significant association with cervical or vulvar cancers before multiple testing correction (Table 4). Only one remained significant after correction for multiple testing: the (T) allele of rs879576 in the coding sequence of the *IL-17RA* gene was associated with HPV16 positive SCC of the cervix (OR 1.91, 95% CI 1.30-2.79; corrected p= 0.018). The full results for genotype associations of all the 560 SNPs typed for candidate genes within the three Th groups can be found in Supplementary Tables 1-5.

## Discussion

In this population-based case-control study, we assessed genetic variation in Th1, Th2 and Th17 immune response associated with HPV-related cervical and vulvar cancers. To our knowledge, this is one of the first investigations of genetic variation in the Th17 genes and cervical and vulvar cancer risk. We also observed associations between SNPs within genes in the Th1 (*CSF2*, *IL-12B* and *TNF*) pathway among subgroups of cervical and vulvar cancer that remained statistically significant even after multiple test correction.

Results from two genome-wide association studies (GWAS) for susceptibility loci for cervical cancer have been recently published.[26, 27] One Swedish study identified three novel independent loci within the major histocompatibility complex (MHC) region contributing to susceptibility to cervical cancers.[27] MHC class I and II molecules play a critical role in antigen presentation to the T-cells during immune response and, therefore, variations in these molecules that are encoded in DNA may affect immune activation against HPV-infected cells.[28] The GWAS results are consistent with the results of a comprehensive study of class I and II alleles in the HLA region for SCC[29] and AC[30] of

the cervix by our group. A different GWAS, conducted in Chinese women, also identified two new loci in the 4q12 and 17q12 regions.[26] The most significant SNP in the 17q12 region (rs8067378) is located near the Gasdermin B (*GSDMB*) gene which is regulated by TGF- $\beta$  signaling.[31] Variation in the TGF- $\beta$  genes was related to cervical and vulvar risk in this study, and are a triggering factor for the Th17 immune response pathway.[32] Together, these studies support further evaluating the role of immune response pathways in relation to cervical and vulvar cancers. Although the two GWAS studies identified loci that are related to immune response, they may have missed identifying important variants due to agnostic approaches that rely on high thresholds for significance. Hence, there is some merit to employing candidate gene approaches that may be helpful in identifying some of these missed associations.

Findings of our study not previously reported included statistically significant association (after correction) for the synonymous (T) allele in SNP rs879576 in the *IL17RA* gene, belonging to the Th17 immune response pathway, with an increased risk for HPV16-positive SCC of the cervix (OR 1.91, 95% CI 1.30-2.79; corrected p= 0.018). In another candidate study, Quan *et al.* evaluated the risk of cervical SCC associated with just two Th17 pathway SNPs: one in the *IL-17A* (rs2275913) and the other in *IL-17F* (rs763780) gene. They reported an increased risk with the *IL-17A* gene polymorphism but not with the *IL-17F* SNP. [33] Genetic variation in Th17 genes has been previously associated with cancers of the breast and the stomach[34, 35], but the exact role of IL-17 and its receptors in the development of cervical cancer has not yet been explored in detail. Our results suggest that genetic variation in the Th17 pathway contributes to cervical carcinogenesis, and that variation in *IL17RA* warrants replication in other populations as it was associated with HPV16-positive squamous cell cancer and adenocarcinoma of the cervix and HPV-positive vulvar cancer. If supported by functional evaluation, this observation may lead to new approaches to immunotherapy for HPV-related cancers.

Previous candidate gene studies have also suggested that genetic polymorphisms in Th1 and Th2 genes may affect susceptibility to HPV infections.[36] Recently, a meta-analysis involving 58 genetic variants in 25 genes, including *IFN-G*, *IL-1B*, *IL-10* and *TNF* genes in Th1 and Th2 pathways, reported significant associations for risk of cervical cancer.[37] A moderately strong association (OR>1.5) was observed for specific polymorphisms in the *IL-1B* and *TNF* genes.[37] Although, these particular SNPs were not evaluated in our study, we did find significant associations with other SNPs in these genes as presented in Table 2. In another study, Chen *et al.*[38] evaluated the risk associated with 3 candidate polymorphisms in the *IL-12A* and *IL-12B* genes and found *IL-12A* rs568408 and *IL-12B* rs3212227 to be significantly associated with cervical cancers in Chinese women. That study had limited power (400 cases; 400 controls) and did not correct for multiple comparisons. In our study, we did not find any significant associations between the *IL-12B* rs3212227 (or rs3212220 which is in linkage disequilibrium with it;  $r^2= 0.95$ ) and cervical cancer risk, although the direction of our results was similar to those from Chen *et al.* A Swedish study also utilized the candidate gene approach to test associations between some SNPs in *IL-4R*, *IL-6* and *IL-10*, and risk of cervical cancer, and found significant associations with a SNP (rs1800797) in the *IL-6* gene without multiple testing correction.[39] Although we did not

test for rs1800797, we evaluated rs1800795 which is in linkage disequilibrium ( $r^2 = 0.97$ ) with rs1800797 and did not find statistically significant associations with cervical or vulvar cancer. Our results, however, were similar in magnitude and direction to the Swedish study. Finally, a Taiwanese study evaluated five candidate polymorphisms in *IL-18* gene and risk of cervical SCC.[40] Similar to our findings, they did not find statistically significant association between SNPs in *IL-18* gene with cervical SCC risk.

There are some limitations to this study. As there were five case groups and 560 SNP evaluated in this study, some of the findings may be attributable to chance. We tried to minimize this possibility by correcting for multiple comparisons and focusing our interpretation on findings that remained statistically significant after correction. As our study was restricted to white women, our results may not be applicable to other racial/ethnic population groups. Further, restriction to white women may not have removed all potential confounding by population stratification. Replication of our results is required in larger studies with higher statistical power, especially for the novel associations within the Th17 pathway. Our study has notable strengths including the population-based recruitment of cases and controls, HPV testing of tumor samples, the ability to focus on HPV16 related tumors, and the tagSNP approach allowed for efficient but representative coverage of each of the selected candidate genes.

In conclusion, our study provides some evidence that common genetic variation in the immune response pathways, particularly Th1 and Th17, may be important in the development and progression of HPV-related cervical and vulvar carcinomas. While these findings are interesting and may point to real signals in the genes studied, there is a possibility that these associations may be proxy for other genes that are in linkage disequilibrium with the candidate genes we examined. Therefore, further studies that involve deeper sequencing or functional evaluation are necessary to assess the impact of genetic variability in immune response pathways in cervical and vulvar cancer etiology.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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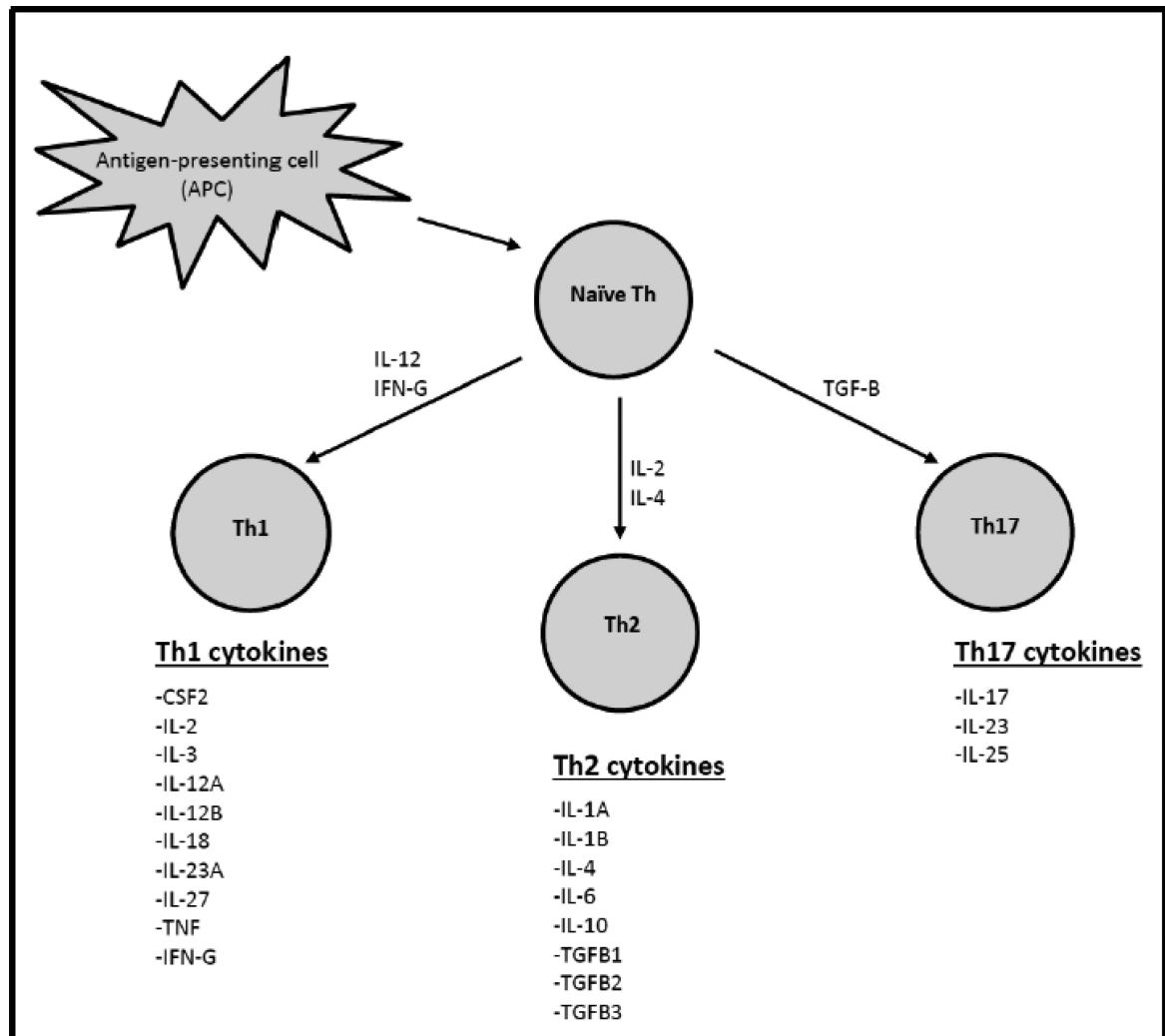
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### Highlights

- Genetic variation in acquired immune response to human papillomavirus (HPV) may contribute to clearance or progression.
- Variation in Th1/Th17 pathway genes were associated with cervical and vulvar cancers, especially with HPV16 positive tumors.
- Novel findings are presented for IL17 and its ligands and their association with cervical and vulvar cancers.



**Figure 1. Cytokines comprising the Th1, Th2 and Th17 immune pathways**

Naïve T-cells may react to interferon-gamma (IFN-G) or interleukin (IL)-12 to differentiate into Th1 cells that clear intracellular pathogens including viruses, or they may respond to IL-4 and IL-2 to evolve into Th2 cells that promote humoral immunity; or transforming growth factor-beta (TGF-β) and IL-6 to promote a Th17 response [Ref: Zhu *et. al*; Cell Research (2010) 20:4-12.].

**Table 1**

Selected characteristics of women with cervical & vulvar cancers, and controls, Seattle-Puget Sound Region, 1986-2004

Characteristic	Controls N = 1109(%)	Cases				
		Squamous cell carcinoma (SCC)			Adenocarcinoma (AC)	
		SCC of the cervix N = 399(%)	HPV16+ SCC <sup>a</sup> of the cervix N= 158 (%)	Vulvar HPV+ SCC N = 357(%)	AC of the cervix N = 502(%)	HPV16+ AC <sup>b</sup> of the cervix N = 136 (%)
Age	45.6 (14.3)	42.6 (11.7)	41.9 (11.3)	47.2 (13.4)	38.4 (10.3)	37.8 (10.5)
Smoking status						
Never	576 (51.9)	150 (37.6)	54 (34.2)	64 (17.9)	266 (53.0)	63 (46.3)
Former	303 (27.3)	109 (27.3)	38 (24.1)	76 (21.3)	130 (25.9)	40 (29.4)
Current	230 (20.7)	140 (35.1)	66 (41.8)	217 (60.8)	106 (21.1)	33 (24.3)
Lifetime sexual partners						
1	295 (26.6)	37 (9.3)	14 (8.9)	24 (6.7)	58 (11.6)	14 (10.3)
2-4	344 (31.0)	122 (30.6)	52 (32.9)	74 (20.7)	134 (26.7)	39 (28.7)
5+	470 (42.4)	240 (60.2)	92 (58.2)	259 (72.6)	310 (61.8)	83 (61.0)
Education						
High school or less	327 (29.5)	157 (39.4)	67 (42.4)	150 (42.0)	126 (25.1)	32 (23.5)
College or more	782 (70.5)	242 (60.7)	91 (57.6)	207 (58.0)	376 (74.9)	104 (76.5)

<sup>c</sup>168 (31.8%) of vulvar cancers did not have a HPV result

<sup>a</sup>118 (29.6%) of squamous cell carcinomas did not have a HPV result

<sup>b</sup>134 (26.7%) of adenocarcinomas did not have a HPV result

**Table 2**

Statistically significant associations between tag SNP's in the Th1 pathway and cervical and vulvar cancer risk, Seattle-Puget Sound Region, 1986-2004

Gene	Site	Cancer type	SNP	Function <sup>d</sup>	Allele	Age-adjusted OR (95% CI)	p-value
CSF2 <sup>a</sup>	Vulva	SCC (HPV+)	rs25882	missense	C/T	1.28(1.04,1.57)	0.0187**
			rs27438	intergenic	A/G	1.26(1.03,1.54)	0.0268**
CSF2RB <sup>a,e</sup>	Cervix	SCC (all subtypes)	rs16997517	missense	C/T	0.37(0.17,0.82)	0.0146
			rs131840	coding sequence	C/T	0.79(0.63,0.97)	0.0273
			rs738149	intronic	A/G	0.79(0.65,0.95)	0.0131
	Vulva	SCC (HPV+)	rs16997517	missense	C/T	0.32(0.14,0.76)	0.0092
			rs738149	intronic	A/G	1.22(1.00,1.48)	0.0457
		rs16845	missense	C/G	0.64(0.42,0.98)	0.0413	
IL1B	Cervix	AC (all subtypes)	rs1071676	UTR-3	C/G	0.80(0.66,0.97)	0.0212
			rs1143634	coding sequence	C/T	0.81(0.67,0.98)	0.027
IL1R1-IL1RL2	Cervix	SCC (all subtypes)	rs3917273	intronic	A/T	0.81(0.69,0.97)	0.0179
			rs7583215	intronic	C/T	1.31(1.06,1.61)	0.0122
IL1R2	Cervix	AC (HPV16 only)	rs2072475	intronic	A/G	0.57(0.36,0.90)	0.0168
			rs2072476	intronic	A/G	0.51(0.29,0.89)	0.0186
			rs719250	intronic	A/G	0.51(0.28,0.90)	0.0216
	Cervix	AC (all subtypes)	rs2072475	intronic	A/G	0.74(0.59,0.93)	0.0095
IL1RN	Cervix	SCC (all subtypes)	rs9005	UTR-3	A/G	0.80(0.66,0.97)	0.0209
IL18	Cervix	SCC (HPV16 only)	rs187238	nearGene-5	C/G	0.75(0.56,1.00)	0.0486
			rs1946519	nearGene-5	A/C	0.75(0.58,0.97)	0.0305
			rs5744222	nearGene-5	A/C	1.32(1.01,1.72)	0.0401
IL18BP	Cervix	AC (HPV16 only)	rs1892919	intronic	C/T	1.71(1.03,2.82)	0.037
IL12A <sup>b,f</sup>	Vulva	SCC (HPV+)	rs2243154	intronic	A/G	1.37(1.04,1.81)	0.0254
IL12B <sup>b,f</sup>	Cervix	AC (all subtypes)	rs10052709	intronic	C/G	0.72(0.56,0.93)	0.0103
			rs2569254 <sup>c</sup>	intronic	C/T	1.23(1.01,1.50)	0.0438
			rs3181225	intergenic	C/T	1.26(1.04,1.53)	0.0202
			rs3213119	missense	G/T	0.52(0.29,0.94)	0.0294
	Vulva	SCC (HPV+)	rs2569254 <sup>c</sup>	intronic	C/T	1.41(1.13,1.76)	0.0024**
rs3181225			intergenic	C/T	1.40(1.13,1.73)	0.0021**	
		rs6870828	intergenic	C/T	1.23(1.03,1.47)	0.0218 <sup>b</sup>	
IL12RB2 <sup>b,f</sup>	Cervix	AC (all subtypes)	rs2307153	missense	A/G	1.78(1.05,3.00)	0.0314
IL2RA <sup>b,g</sup>	Cervix	SCC (all subtypes)	rs2228150	coding sequence	A/G	1.91(1.22,2.99)	0.0047
			rs3134883	intronic	C/T	1.20(1.00,1.44)	0.0462
	Cervix	SCC (HPV16 only)	rs2228150	coding sequence	A/G	2.11(1.15,3.86)	0.0152
			rs10905656	intronic	A/C	0.71(0.55,0.92)	0.0106
		rs12722516	intronic	C/T	1.96(1.11,3.45)	0.0202	

Gene	Site	Cancer type	SNP	Function <sup>d</sup>	Allele	Age-adjusted OR (95% CI)	p-value
			rs6602379	intronic	A/G	0.71(0.54,0.94)	0.0174
			rs7072398	intronic	A/G	0.74(0.58,0.95)	0.0196
			rs7910961	intronic	C/T	0.68(0.51,0.90)	0.0066
<i>g</i>	Cervix	AC (all subtypes)	rs12722588	intronic	A/G	1.41(1.03,1.93)	0.033
			rs1323658	intronic		0.36(0.15,0.84)	0.0185
			rs2104286	intronic	A/G	1.33(1.01,1.76)	0.0457
	Cervix	AC (HPV16 only)	rs10905669	intronic	C/T	1.24(1.01,1.51)	0.0351
			rs12722486	intronic	A/G	0.59(0.37,0.94)	0.0274
IL2RB <sup>b</sup>	Cervix	AC (HPV16 only)	rs3218253	intronic	C/T	1.34(1.01,1.77)	0.0435
IL3 <sup>a</sup>	Vulva	SCC (HPV+)	rs181781	nearGene-5	A/G	1.43(1.07,1.91)	0.0142
			rs31481	intronic	A/G	1.33(1.07,1.66)	0.0119
			rs40401	missense	C/T	1.29(1.05,1.58)	0.0133
IFNG	Cervix	AC (all subtypes)	rs2069716	intronic	A/G	0.66(0.45,0.97)	0.0368
TNF <sup>b,h</sup>	Cervix	SCC (all subtypes)	rs1052248	nearGene-3	A/T	0.74(0.61,0.89)	0.0019**
			rs1799964	nearGene-5	C/T	0.65(0.52,0.81)	0.0001**
			rs2009658	nearGene-5	C/G	0.63(0.49,0.81)	0.0003**
			rs2229094	missense	C/T	0.66(0.54,0.81)	0.0001**
			rs2239704	UTR-5	G/T	1.31(1.10,1.55)	0.0019**
			rs2256965	intronic	A/C/T	1.22(1.04,1.44)	0.0174
			rs3093662	intronic	A/G	0.69(0.48,0.98)	0.0381
			rs915654	nearGene-5	A/T	0.81(0.68,0.97)	0.0192
	Cervix	SCC (HPV16 only)	rs1052248	nearGene-3	A/T	0.70(0.53,0.93)	0.0149
			rs1799964	nearGene-5	C/T	0.65(0.47,0.90)	0.0091
			rs2009658	nearGene-5	C/G	0.57(0.38,0.87)	0.0085
			rs2229094	missense	C/T	0.61(0.45,0.83)	0.0017**
			rs2239704	UTR-5	G/T	1.35(1.05,1.72)	0.0179
			rs915654	nearGene-5	A/T	0.74(0.57,0.97)	0.0288
<i>h</i>	Cervix	AC (all subtypes)	rs1799964	nearGene-5	C/T	0.80(0.66,0.97)	0.0261
			rs2229094	missense	C/T	0.80(0.67,0.96)	0.0172
			rs2239704	UTR-5	G/T	1.30(1.10,1.52)	0.0016**
			rs2256965	intronic	A/C/T	1.20(1.03,1.41)	0.0199
			rs915654	nearGene-5	A/T	0.77(0.65,0.91)	0.0019**
	Cervix	AC (HPV16 only)	rs2256965	intronic	A/C/T	1.34(1.04,1.73)	0.0241
			rs915654	nearGene-5	A/T	0.75(0.57,0.99)	0.0455
<i>h</i>	Vulva	SCC (HPV+)	rs1052248	nearGene-3	A/T	0.74(0.60,0.90)	0.0028**
			rs1799964	nearGene-5	C/T	0.77(0.62,0.96)	0.021
			rs2009658	nearGene-5	C/G	0.71(0.54,0.93)	0.0137



Gene	Site	Cancer type	SNP	Function <sup>d</sup>	Allele	Age-adjusted OR (95% CI)	p-value
			rs2229094	missense	C/T	0.71(0.58,0.88)	0.0013**
			rs2239704	UTR-5	G/T	1.58(1.33,1.88)	<0.0001**
			rs2256965	intronic	A/C/T	1.49(1.25,1.77)	<0.0001**
			rs915654	nearGene-5	A/T	0.65(0.53,0.78)	<0.0001**
			rs1800610	intronic	C/T	1.52(1.11,2.08)	0.0093
			rs769178	intergenic	A/C	1.65(1.20,2.26)	0.0019**
TNFRSF1A <sup>b,h</sup>	Cervix	SCC (all subtypes)	rs4149584	missense	A/C/G/T	0.44(0.20,0.99)	0.0481
	Cervix	SCC (HPV16 only)	rs1800693	intronic	A/G	1.33(1.04,1.70)	0.0242
			rs4149575	intronic	A/C	0.55(0.32,0.95)	0.0322
			rs767455	coding sequence	C/T	1.30(1.01,1.66)	0.0386
TNFRSF1B <sup>b</sup>	Cervix	AC (HPV16 only)	rs1061631	UTR-3	A/G	1.50(1.11,2.03)	0.0078
			rs1148458	intronic	C/T	1.62(1.01,2.60)	0.0469
			rs976881	intronic	A/G	1.38(1.06,1.81)	0.0183
<i>h</i>	Vulva	SCC (HPV+)	rs1061631	UTR-3	A/G	1.24(1.01,1.53)	0.0443
			rs976881	intronic	A/G	1.21(1.01,1.45)	0.0377
			rs17882988	intronic	A/G	1.26(1.00,1.58)	0.0466
			rs499646	intronic	C/T	0.62(0.39,0.97)	0.0365
			rs652625	nearGene-5	A/T	0.65(0.43,0.99)	0.0463

Highlighted portions indicate previously published results from our study population; references are noted for prior reports.

\*\* Significant after correction for multiple testing using Holm's stepdown procedure

<sup>a</sup> Results for squamous cell carcinoma of the cervix previously published by our group

<sup>b</sup> Results for squamous cell carcinoma (all), adenocarcinoma (all) and HPV+ vulvar cancer previously published by our group

<sup>c</sup> rs2569254 had 6.1% genotype data

<sup>d</sup> intronic denotes that the SNP is located in the intronic region of the gene (except in the first 2 or last 2 bases), intergenic denotes that the SNP is located in between two genes, nearGene-3 denotes the SNP is located near the 3' end, nearGene-5 denotes the SNP is located near the 5' end, UTR-3 denotes that the SNP is located in the untranslated 3' region, UTR-5 denotes that the SNP is located in the untranslated 5' region, missense denotes that the SNP is present in the coding region and causes a change in the coded peptide, and coding sequence denotes that the SNP is present in the coding region and does not cause a change in the peptide coded.

<sup>e</sup> See reference 17 (Johnson et. al.)

<sup>f</sup> See reference 18 (Hussain et. al.)

<sup>g</sup> See reference 19 (Hussain et. al.)

<sup>h</sup> See reference 16 (Bodelon et. al.)

**Table 3**

Statistically significant associations between tag SNP's in the Th2 pathway and cervical and vulvar cancer risk, Seattle-Puget Sound Region, 1986-2004

Gene	Site	Cancer type	SNP	Function	Allele	Age-adjusted OR (95% CI)	p-value
IL4R	Vulva	SCC (HPV+)	rs3024668	nearGene-5	A/G	0.59(0.37,0.95)	0.0304
IL6	Cervix	SCC (all subtypes)	rs2069840	Intronic	C/G	1.25(1.05,1.49)	0.0137
		SCC (HPV16 only)	rs2069840	Intronic	C/G	1.32(1.02,1.70)	0.0327
				rs2069837	Intronic	A/G	0.42(0.21,0.81)
IL10 <sup>a</sup>	Cervix	SCC (HPV16 only)	rs3024509	Intronic	C/T	0.49(0.26,0.93)	0.0293
		AC (HPV16 only)	rs3024509	Intronic	C/T	0.48(0.24,0.95)	0.0337
				rs1800894	nearGene-5	A/G	0.34(0.12,0.94)
IL10RA <sup>a,c</sup>	Cervix	AC (all subtypes)	rs2229113 <sup>b</sup>	Missense	A/G	1.21(1.02,1.43)	0.0304
			rs2256111	coding sequence	A/G	1.19(1.01,1.39)	0.0326
			rs2512143	Intronic	A/G	1.23(1.05,1.45)	0.0129
			rs2512148	Intergenic	A/C	1.22(1.04,1.44)	0.0172
IL10RB <sup>a</sup>	Cervix	AC (HPV16 only)	rs2843701	Intronic	C/T	0.75(0.57,0.98)	0.0327
TGFB1	Cervix	SCC (all subtypes)	rs11466338	Intronic	A/G	1.48(1.05,2.09)	0.0243
TGFB2	Cervix	SCC (HPV16 only)	rs17047804	Intronic	C/T	1.55(1.06,2.26)	0.0237
			rs2000220	Intronic	A/G	1.29(1.01,1.65)	0.0407
		Cervix	AC (all subtypes)	rs4846476	Intronic	C/G	0.81(0.67,0.99)
TGFB3	Cervix	SCC (all subtypes)	rs3917148	Intronic	A/C	1.36(1.00,1.85)	0.0494
	Vulva	SCC (HPV+)	rs3917200	Intronic	C/T	1.44(1.03,2.01)	0.0347
TGFB1	Cervix	SCC (all subtypes)	rs10512263	Intronic	C/T	0.66(0.46,0.94)	0.0226
	Cervix	SCC (HPV16 only)	rs10512263	Intronic	C/T	0.51(0.29,0.91)	0.0236
TGFB2	Cervix	AC (HPV16 only)	rs9858487	Intronic	C/G	1.69(1.00,2.83)	0.0481
	Vulva	SCC (HPV+)	rs3087465	nearGene-5	A/G	1.23(1.00,1.51)	0.0451

Highlighted portions indicate previously published results from our study population.

<sup>a</sup>Results for squamous cell carcinoma (all), adenocarcinoma (all) and HPV+ vulvar cancer previously published by our group

<sup>b</sup>rs2229113 had 5.1% missing genotype data

<sup>c</sup>See reference 18 (Hussain et. al.)

**Table 4**

Statistically significant associations between tag SNP's in the Th17 pathway and cervical and vulvar cancer risk, Seattle-Puget Sound Region, 1986-2004

Gene	Site	Cancer type	SNP	Function	Allele	Age-adjusted OR (95% CI)	p-value
IL17A	Cervix	SCC (all subtypes)	rs8193036	nearGene-5	C/T	0.80(0.66,0.97)	0.0231
	Cervix	SCC (HPV16 only)	rs8193036	nearGene-5	C/T	0.74(0.55,1.00)	0.0473
IL17F	Cervix	SCC (all subtypes)	rs11465549	intronic	A/G	1.72(1.07,2.77)	0.0265
	Cervix	AC (all subtypes)	rs12201582	intronic	A/C	1.26(1.03,1.54)	0.0219
	Cervix	AC (HPV16 only)	rs11465549	intronic	A/C	2.09(1.04,4.22)	0.0395
	Vulva	SCC (HPV+)	rs11465549	intronic		1.63(1.00,2.65)	0.0489
IL17RA	Cervix	SCC (all subtypes)	rs2241049	intronic coding	A/G	0.83(0.70,0.99)	0.0419
			rs879576	sequence	C/T	1.38(1.05,1.83)	0.0225
	Cervix	SCC (HPV16 only)	rs5992628	UTR-3	G/T	0.77(0.60,0.99)	0.0408
			rs879574	intronic coding	A/T	1.59(1.09,2.34)	0.0172
			rs879576	sequence	C/T	1.91(1.30,2.79)	0.001**
			rs4819956	intergenic coding	A/C	1.27(1.07,1.50)	0.0065
	Cervix	AC (all subtypes)	rs879576	sequence	C/T	1.31(1.03,1.67)	0.0289
			rs17807076	intronic	C/T	0.64(0.47,0.88)	0.0065
	Cervix	AC (HPV16 only)	rs4819956	intergenic coding	A/C	1.37(1.04,1.80)	0.026
			rs879576	sequence	C/T	1.57(1.03,2.41)	0.0361
rs879574			intronic	A/T	1.31(1.01,1.70)	0.0402	
rs879576			sequence	C/T	1.57(1.03,2.41)	0.0361	
Vulva	SCC (HPV+)	rs879574	intronic	A/T	1.31(1.01,1.70)	0.0402	
		rs879576	sequence	C/T	1.57(1.03,2.41)	0.0361	
IL23R	Cervix	AC (all subtypes)	rs6588250	intronic	G/T	1.21(1.01,1.44)	0.0386
			rs7528924	intronic	A/G	1.23(1.03,1.48)	0.0258
			rs790633	intronic	C/T	1.21(1.03,1.44)	0.0243
	Cervix	AC (HPV16 only)	rs6693831	intronic	C/T	1.42(1.06,1.89)	0.0182
	Vulva	SCC (HPV+)	rs10889675	intronic	A/C	1.39(1.09,1.78)	0.008
			rs6693831	intronic	C/T	1.30(1.07,1.58)	0.0084

Highlighted portions indicate previously published results from our study population.

\*\* Significant after correction for multiple testing using Holm's stepdown procedure