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Genetic variation in the TLR and NF-κB genetic pathways and cervical and vulvar cancer risk: a population-based case-control study

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Abstract

Genital infection with the oncogenic human papillomavirus (HPV) is the necessary cause of cervical cancer and of a large fraction of vulvar cancers. The toll-like receptor (TLR) and the nuclear factor κB (NF-κB) signaling pathways have been implicated in inflammation, autoimmune disease and cancer, but whether common nucleotide variation in these pathways is associated with the risk of cervical and vulvar cancers has received little study. Using data from a population-based case-control study of cervical and vulvar cancers, we genotyped 205 single nucleotide polymorphisms (SNPs) in and around 32 candidate gene regions within these pathways. Gene-based analyses were employed to estimate the associations between individual gene regions and the risk of cervical and vulvar cancers. Odds ratio (OR) and 95% confidence intervals (CI) were calculated to assess the risk of cervical and vulvar cancers for each SNP. P-values were adjusted for multiple testing. A total of 876 cervical cancer cases, 517 vulvar cancer cases and 1,100 controls were included in the analysis. The TNF region was significantly associated with the risks of cervical cancer (gene-based P-value: 2.0×10^{-4}) and vulvar cancer (gene-based P-value: 1.0×10^{-4}). The rare allele (A) of SNP rs2239704 in the 5′ UTR of the LTA gene was significantly associated with increased risks of cervical cancer (OR=1.31, 95% CI: 1.15–1.50; adjusted P-value: 0.013) and vulvar cancer (OR=1.51, 95% CI: 1.30–1.75; adjusted P-value: 1.9×10^{-5}). These findings add to the evidence of the importance of the immune system in the etiology of cervical and vulvar cancers.

Keywords

cervical cancer; vulvar cancer; toll-like receptor; nuclear factor-κB; tumor necrosis factor

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Introduction

Genital infection with the oncogenic human papillomavirus (HPV) is the necessary cause of cervical cancer and of a large fraction of vulvar cancers[1,2]. Although HPV is a common sexually transmitted infection[3,4], most women exposed to HPV do not develop cervical or vulvar cancer[5], suggesting that other factors must be important for the establishment of a persistent infection that can lead to these cancers. Several inherited genetic factors that influence the host immune response in the presence of HPV have been reported to confer susceptibility to these anogenital cancers[6].

Toll-like receptors (TLRs) are transmembrane proteins that recognize specific pathogen-associated molecular patterns (PAMPs) found in viruses and other invading pathogens[7,8]. Different TLRs are able to identify different PAMPs. Upon activation of the TLRs, a signal transduction is initiated, which involves several adaptor proteins (including TIRAP, TOLLIP, TICAM1 and TICAM2) and several activator proteins (including TANK, IRAK1, IRAK4, TBK1 and IKBKE). This signaling cascade promotes the binding of the nuclear factor-κB (NF-κB) to DNA, which in turn induces cytokine transcription, essential to induce an immune response. An alternative pathway of activation of NF-κB is through the tumor necrosis factor (TNF) superfamily[9–11]. TNF exercises its function by binding to a member of the TNF receptor superfamily, which includes TNFRSF1A and TNFRSF1B, and the recruitment of several members of the TNF receptor-associated factors (TRAFs) family, such as TANK or TRAF6.

TLRs are thought to be involved in the response to HPV infection[12–14]. TLR genes are expressed in the female genital tract[12,15] and there is some evidence that they may play a role in the clearance of the HPV infection[14]. HPV oncoproteins E6 and E7 have also been observed to alter the expression of several components of the NF-κB signal pathway in cervical keranocytes[16]. However, whether the TLR and NF-κB pathways are associated with the risks of cervical and vulvar cancers has not been adequately studied. Therefore, we examined the associations between the common genetic variation in the TLR and NF-κB genetic pathways and cervical and vulvar carcinomas, using data from a population-based case-control study.

Methods

Study Design and Population

The study population and collection of specimens have been described previously[17]. Briefly, cervical cancer cases were women newly diagnosed with invasive squamous cell carcinoma (SCC) of the cervix (ICD-0 8010–8081) or invasive and *in situ* adenocarcinoma of the cervix (ICD-0 8140–8560) between January 1986 and June 1998 or between January 2000 and December 2004. They were identified through the Cancer Surveillance System (CSS), a population-based tumor registry that is part of the Surveillance, Epidemiology and End Results program of the National Cancer Institute[18]. Similarly, vulvar cancer cases were women newly diagnosed with invasive or *in situ* SCC of the vulva (ICD-0 8010, 8070–8077, 8081) during the same period as the cervical cases and also identified through the CSS. Controls were women without a history of cervical or vulvar cancers, matched to cases on 5-year age groups and county of residency. They were identified by random digit telephone dialing. All women in the study were aged 18–74 years old at the time of diagnosis for the cases or the reference date for the controls. The Institutional Review Board of the Fred Hutchinson Cancer Research Center approved the study.

Data and Specimen collection

In-person interviews were conducted to obtain information on demographic characteristics, reproductive and smoking history and family history of cancers. Blood or buccal samples were collected at the time of the interview.

Genomic DNA was extracted from buffy coats from blood samples or from cell pellets from buccal samples. Archival tissue blocks from biopsy or surgery were retrieved to determine the presence and type of HPV DNA in the tumors of cervical and vulvar cases using a polymerase chain reaction (PCR)[19].

SNP Selection and Genotyping

Thirty-two candidate genes related to the TLR and NF-κB genetic pathways were included in this study (AZI2, IKBKE, IRAK1, IRAK4, IRF3, LST1, LTA, LTB, MAP3K1, MAP3K7, NCR3, NFKB1, NFKB2, RELA, RELB, TANK, TBK1, TICAM1, TICAM2, TIRAP, TLR3, TLR4, TLR7, TLR9, TNF, TNFRSF1A, TNFRSF1B, TOLLIP, TRAF3, TRAF6, VISA and ZBP1). Tagging single nucleotide polymorphisms (tagSNPs) covering the exons, introns and the 3′ and 5′ 4kb flanking sequences were selected for each of the above genes. Because of the proximity of genes LST1, LTA, LTB, NCR3 and TNF and the fact that the flanking sequences overlap, they were collectively named the TNF-region. Using information from the CEU-HapMap population, the SeattleSNPs Variation Discovery Resource (http://gvs.gs.washington.edu) and the Snagger algorithm[20], tagSNPs were selected based on having a pairwise $r^2 \geq 0.80$ and minor allele frequency (MAF) greater than 5% for each gene.

A total of 261 tagSNPs were genotyped using the Illumina Goldengate multiplex platform (Illumina Inc., San Diego, USA) or the KASPar platform (KBioscience, Hoddesdon, UK). Forty-two tagSNPs failed genotyping and 5 were monomorphic. Of the remaining 214 tagSNPs, 9 tagSNPs had MAF less than 1% in our control population and were excluded from the analysis, leaving 205 tagSNPs in 28 candidate gene regions (Supplemental Table 1).

Data Analysis

Analyses were restricted to self-reported Caucasian subjects (88.8% of cervical cases, 95.1% of vulvar cases and 91.8% of controls). We also excluded women with zero lifetime sex partners (0.2% of cervical cases, 0.2% of vulvar cases and 1.3% of controls) so that women could have been exposed to oncogenic HPV. Hardy-Weinberg equilibrium was calculated among controls using an exact test[21] (Supplemental Table 1). After correcting for multiple testing, none of the tagSNP genotypes showed evidence of deviation from Hardy-Weinberg equilibrium. Linkage disequilibrium (LD) was estimated in our control population using the r^2 measure among the 205 tagSNPs (Supplemental Figure 1). Associations between gene regions and disease outcome were computed by combining SNP-based p-values by means of an adaptive rank truncated product[22]. This method takes into account the LD structure of the SNPs within a gene region to compute the gene-based p-value. Significance of the gene-based P-value was assessed using a permutation procedure with 10,000 permutations. SNP-based p-values, odds ratios (ORs) and 95% confidence intervals (CIs) for the associations between SNPs and cancer were computed using unconditional logistic regression assuming an additive model (coded as 0, 1, 2) and adjusted for age as a continuous variable. Due to X inactivation, analyses of tagSNPs in the X chromosome were restricted to women with homozygous genotypes. Adjusted p-values were gene-based p-values or SNP-based p-values adjusted for multiple testing using the Bonferroni method. Analyses were carried out using the R software environment (version 2.15.0 for Macintosh).

Results

A total of 876 cervical cases, 517 vulvar cases and 1,100 controls were included in the analysis. Of the 876 cervical cases, 403 (46%) were diagnosed with SCC and 473 (54%) with adenocarcinoma. Of the 517 women with vulvar cancer, 429 (83%) were tested for HPV DNA, with the majority of them testing positive (350, 82%) and only a small fraction testing negative (79, 18%). Cervical cases were younger, current smokers and had more lifetime sex partners compared to controls (Supplemental Table 2). Vulvar cases were slightly older, more likely to be current smokers, had less education, and a higher number of lifetime sex partners than controls.

Gene-based analysis identified the TNF region to be significantly associated with the risks of cervical cancer (gene-based P-value: 2.0×10^{-4}; Table 1) and vulvar cancer (gene-based P-value: 1.0×10^{-4}). The association remained significant after Bonferroni adjustment for multiple testing and further adjustment for smoking and lifetime number of sex partners (results not shown). When analyses were stratified by histological types, the TNF region was still significantly associated with the risk of cervical SCC (gene-based P-value: 1.5×10^{-3}) and with HPV positive vulvar cancer (gene-based P-value: 1.0×10^{-4}). Other gene regions did not reach statistical significance after correcting for multiple testing.

Of the twelve tagSNPs in the TNF region, several were associated with the risks of cervical and vulvar cancers. The rare allele (A) of the LTA (lymphotoxin alpha) rs2239704 5′ untranslated region (UTR) tagSNP was significantly associated with a 31% increased risk of cervical cancer (OR=1.31, 95% CI: 1.15–1.50; adjusted P-value: 0.013; Table 2) and with a 51% increased risk of vulvar cancer (OR=1.51, 95% CI: 1.30–1.75; adjusted P-value: 1.9×10^{-5}; Table 3). When cervical cancer was stratified by histological type, there was still a 31% increased risk for SCC (OR=1.31, 95% CI: 1.11–1.55; adjusted P-value: 0.342) and adenocarcinoma (OR=1.31, 95% CI: 1.11–1.54; adjusted P-value: 0.298). When vulvar cancer was stratified by HPV status, the A allele of SNP rs2239704 was associated with a significant 58% increased risk of HPV-positive vulvar cancer (OR=1.58, 95% CI: 1.33–1.87; adjusted P-value: 5.1×10^{-5}), while it was associated with a non-significant 24% increased risk of HPV-negative vulvar cancer (OR=1.24, 95% CI: 0.89–1.73; adjusted P-value: 1.00).

The rare allele (G) of LTA the rs2229094 missense tagSNP was significantly associated with a decreased risk of cervical cancer (OR=0.72, 95% CI: 0.62–0.84; adjusted P-value: 0.005). This significant association was present for cervical SCC (OR=0.72, 95% CI: 0.62–0.84; adjusted P-value: 0.005). This allele was also associated with a decreased risk of cervical adenocarcinoma (OR=0.77, 95% CI: 0.64–0.93; adjusted P-value: 1.000), vulvar cancer (OR=0.74, 95% CI: 0.62–0.88; adjusted P-value: 0.172), and HPV positive vulvar cancer (OR=0.71, 95% CI: 0.58–0.87; adjusted P-value: 0.216), albeit not significant after correcting for multiple testing. Similar associations were found for the rare allele (G) of the TNF rs1799964 flanking 5′ UTR tagSNP. The pairwise LD between tagSNPs rs2229094 and rs1799964 was $r^2=0.76$.

The minor allele (A) of LTA rs915654 flanking 5′ UTR tagSNP was significantly associated with a decreased risk of vulvar cancer (OR=0.70, 95% CI: 0.59–0.72; adjusted P-value: 3.2×10^{-3}) and of HPV positive vulvar cancer (OR=0.65, 95% CI: 0.54–0.79; adjusted P-value: 2.2×10^{-3}). The strength of the association was weaker for HPV negative vulvar cancer and cervical cancer (Tables 2 and 3).

Finally, the minor allele (A) of the LST1 (leukocyte specific transcript 1) rs2256965 intronic tagSNP was significantly associated with an increased risk of vulvar cancer (OR=1.43, 95% CI: 1.23–1.66; adjusted P-value: 5.5×10^{-4}) and of HPV positive vulvar cancer (OR=1.48,

95% CI: 1.25–1.76; adjusted P-value: 1.4×10^{-3}). The increased risk was also present for HPV negative vulvar cancer and cervical cancer (overall and for SCC and adenocarcinoma), but they did not reach statistical significance after Bonferroni correction (Tables 2 and 3).

Associations between other SNPs and the risks of cervical and vulvar cancers are presented in Supplemental Tables 3–8.

Discussion

In this study of the TLR and NF-κB genetic pathways on the risk of cervical and vulvar cancers, we found some evidence that common variation in the TNF region was significantly associated with the risks of both types of malignancies. In particular, within the TNF-region, we found that the SNP rs2239704, located in the 5′ UTR of the LTA gene, was significantly associated with increased risks of cervical and vulvar cancers.

The TNF gene region is a rich gene locus that includes LTA upstream of TNF and LTB (lymphotoxin beta), LST1 and NCR3 (natural cytotoxicity triggering receptor 3) downstream of TNF. LTA and LTB belong to the TNF superfamily (members 1 and 3, respectively) and reside in the major histoccompatiblity complex (MHC) on chromosome 6, variation in which has also been found to be associated with cervical cancer[23–25]. To better understand the genetic relationship between the TNF-region and other areas within the MHC region, we computed the LD structure between tagSNPs in five major loci within the MHC (HLA-A, HLA-B, HLA-C, HLA-DRB1 and HLA-DQB1) and the SNPs in the TNF-region presented in our study using data from 40 women with European ancestry downloaded from the 1000 genomes (http://www.1000genomes.org) (Supplemental Figure 2). There was little evidence of high linkage between the tagSNPs in the MHC and the tagSNPs in the TNF-region analyzed (all pairs between the two regions had $r^2<0.379$). However, this was a small dataset and information on some MHC tagSNPs was not available in the 1000 genomes dataset. In addition, most HLA alleles are not well captured using tagSNPs, and a more focused studied is needed.

LTA codes for a cytokine produced by lymphocytes and plays an important role in the genesis of lymphoid organs[26]. Genetic variation at the LTA/TNF/LTB locus has been associated with several infectious diseases, such as the denge virus[27], malaria [28], influenza [29] or sepsis [30] as well as with non-Hodgkin's lymphoma [31,32].

In contrast to our findings, a previous study reported that 5′ UTR SNP rs2239704 was not associated with HPV persistence or progression to cervical cancer in a Costa Rican population[33]. This could be due to the lower power in the previous study (469 women with cancer, 390 with persistent HPV infection and 452 controls), to the ethnically distinct study populations, or to the potential involvement of this SNP in the initial response to infection, rather than at later stages of carcinogenesis.

The G allele of rs2229094, a missense mutation Cys13Arg in the LTA gene, was significantly associated with a 30% decreased risk of cervical cancer in our study. Although the association was not found to be significant with vulvar cancer, it was of the same magnitude and direction. This SNP was found to be associated with cancer-specific mortality in a recent prospective study [34]. In addition, SNP rs1799964, which is in moderate to high LD with rs2229094 ($r^2=0.76$ in our control population), has also been inversely related to smoking related cancers[35], and cigarette smoking is a risk factor for SCC cervical and vulvar cancers.

Finally, the G allele of rs2256965, a SNP located in an intronic region of the LST1 gene, was significantly associated with a 40% increased risk of vulvar cancer, but to a lesser

extent with cervical cancer. The protein encoded by the LST1 gene can inhibit the proliferation of lymphocytes, potentially preventing a competent immune response to the HPV virus. This gene, in combination with HLA genes, has been found to be important in the immune response to rubella vaccine [36].

The role of genetic variation in the TLR genes on the risk of cervical or vulvar cancer has not been well-studied. We did not observe any significant association between tagSNPs in TLR coding genes and the risk of these cancers. A prior study looked at two SNPs on TLR4 in relation to persistence or progression of cervical cancer but did not report assocations[33]. However, the same study also found IRF3 SNP rs7251 to be associated with persistence, which were not associated with cervical and vulvar risk in our study.

The main limitation of our study was the relatively small number of cases and controls which could have prevented us from detecting weaker associations between SNPs and cervical or vulvar cancer. Due to the large number of SNPs that we studied, some of the reported associations could have been due to chance. However, we corrected for multiple testing and focused our discussion on the gene region that was associated with both HPV-related cancers. Further, the individual SNPs reported here had similar associations, in direction and magnitude, for cervical and vulvar cancers. A strength of our study was the use of gene-set analysis that allowed us to maximize our ability to detect effects that are only significant after combining the small effects of individual SNPs.

In summary, our study underscores the role of genetic variation of immune genes in the development of HPV related cervical and vulvar cancers. Future studies should further examine the role of the TNF region in the cervical and vulvar cancer etiology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, Snijders PJ, Peto J, Meijer CJ, Munoz N. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol. 1999; 189:12–9. [PubMed: 10451482]
2. De Vuyst H, Clifford GM, Nascimento MC, Madeleine MM, Franceschi S. Prevalence and type distribution of human papillomavirus in carcinoma and intraepithelial neoplasia of the vulva, vagina and anus: a meta-analysis. Int J Cancer. 2009; 124:1626–36. [PubMed: 19115209]
3. Lindau ST, Drum ML, Gaumer E, Surawska H, Jordan JA. Prevalence of high-risk human papillomavirus among older women. Obstet Gynecol. 2008; 112:979–89. [PubMed: 18978096]
4. Hariri S, Unger ER, Sternberg M, Dunne EF, Swan D, Patel S, Markowitz LE. Prevalence of genital human papillomavirus among females in the United States, the National Health And Nutrition Examination Survey, 2003–2006. J Infect Dis. 2011; 204:566–73. [PubMed: 21791659]
5. Schiffman M, Castle PE, Jeronimo J, Rodriguez AC, Wacholder S. Human papillomavirus and cervical cancer. Lancet. 2007; 370:890–907. [PubMed: 17826171]
6. Magnusson PK, Lichtenstein P, Gyllensten UB. Heritability of cervical tumours. Int J Cancer. 2000; 88:698–701. [PubMed: 11072236]

7. Rassa JC, Ross SR. Viruses and Toll-like receptors. Microbes Infect. 2003; 5:961–8. [PubMed: 12941388]
8. Akira S, Takeda K. Toll-like receptor signalling. Nat Rev Immunol. 2004; 4:499–511. [PubMed: 15229469]
9. Baeuerle PA, Henkel T. Function and activation of NF-kappa B in the immune system. Annu Rev Immunol. 1994; 12:141–79. [PubMed: 8011280]
10. Aggarwal BB. Signalling pathways of the TNF superfamily: a double-edged sword. Nat Rev Immunol. 2003; 3:745–56. [PubMed: 12949498]
11. Verstrepen L, Bekaert T, Chau TL, Tavernier J, Chariot A, Beyaert R. TLR-4, IL-1R and TNF-R signaling to NF-kappaB: variations on a common theme. Cell Mol Life Sci. 2008; 65:2964–78. [PubMed: 18535784]
12. Pioli PA, Amiel E, Schaefer TM, Connolly JE, Wira CR, Guyre PM. Differential expression of Toll-like receptors 2 and 4 in tissues of the human female reproductive tract. Infect Immun. 2004; 72:5799–806. [PubMed: 15385480]
13. Yan M, Peng J, Jabbar IA, Liu X, Filgueira L, Frazer IH, Thomas R. Activation of dendritic cells by human papillomavirus-like particles through TLR4 and NF-kappaB-mediated signalling, moderated by TGF-beta. Immunol Cell Biol. 2005; 83:83–91. [PubMed: 15661045]
14. Daud II, Scott ME, Ma Y, Shiboski S, Farhat S, Moscicki AB. Association between toll-like receptor expression and human papillomavirus type 16 persistence. Int J Cancer. 2011; 128:879–86. [PubMed: 20473890]
15. Nasu K, Narahara H. Pattern recognition via the toll-like receptor system in the human female genital tract. Mediators Inflamm. 2010; 2010:976024. [PubMed: 20396665]
16. Nees M, Geoghegan JM, Hyman T, Frank S, Miller L, Woodworth CD. Papillomavirus Type 16 oncogenes downregulate expression of interferon-responsive genes and upregulate proliferation-associated and NF-kB-responsive genes in cervical keratinocytes. Journal of Virology. 2001; 75:4283–96. [PubMed: 11287578]
17. Bodelon C, Madeleine MM, Johnson LG, Du Q, Malkki M, Petersdorf EW, Schwartz SM. Genetic variation in CD83 and risks of cervical and vulvar cancers: a population-based case-control study. Gynecol Oncol. 2012; 124:525–8. [PubMed: 22134374]
18. Hankey BF, Ries LA, Edwards BK. The surveillance, epidemiology, and end results program: a national resource. Cancer Epidemiol Biomarkers Prev. 1999; 8:1117–21. [PubMed: 10613347]
19. Madeleine MM, Anttila T, Schwartz SM, Saikku P, Leinonen M, Carter JJ, Wurscher M, Johnson LG, Galloway DA, Daling JR. Risk of cervical cancer associated with Chlamydia trachomatis antibodies by histology, HPV type and HPV cofactors. Int J Cancer. 2007; 120:650–5. [PubMed: 17096345]
20. Edlund CK, Lee WH, Li D, Van Den Berg DJ, Conti DV. Snagger: a user-friendly program for incorporating additional information for tagSNP selection. BMC Bioinformatics. 2008; 9:174. [PubMed: 18371222]
21. Wigginton JE, Cutler DJ, Abecasis GR. A note on exact tests of Hardy-Weinberg equilibrium. Am J Hum Genet. 2005; 76:887–93. [PubMed: 15789306]
22. Yu K, Li Q, Bergen AW, Pfeiffer RM, Rosenberg PS, Caporaso N, Kraft P, Chatterjee N. Pathway analysis by adaptive combination of P-values. Genet Epidemiol. 2009; 33:700–9. [PubMed: 19333968]
23. Hildesheim A, Wang SS. Host and viral genetics and risk of cervical cancer: a review. Virus Res. 2002; 89:229–40. [PubMed: 12445662]
24. Madeleine MM, Brumback B, Cushing-Haugen KL, Schwartz SM, Daling JR, Smith AG, Nelson JL, Porter P, Shera KA, McDougall JK, Galloway DA. Human leukocyte antigen class II and cervical cancer risk: a population-based study. J Infect Dis. 2002; 186:1565–74. [PubMed: 12447731]
25. Madeleine MM, Johnson LG, Smith AG, Hansen JA, Nisperos BB, Li S, Zhao LP, Daling JR, Schwartz SM, Galloway DA. Comprehensive analysis of HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQB1 loci and squamous cell cervical cancer risk. Cancer Res. 2008; 68:3532–9. [PubMed: 18451182]

26. Elewaut D, Ware CF. The unconventional role of LT alpha beta in T cell differentiation. Trends in immunology. 2007; 28:169–75. [PubMed: 17336158]
27. Vejbaesya S, Luangtrakool P, Luangtrakool K, Kalayanarooj S, Vaughn DW, Endy TP, Mammen MP, Green S, Libraty DH, Ennis FA, Rothman AL, Stephens HA. TNF and LTA gene, allele, and extended HLA haplotype associations with severe dengue virus infection in ethnic Thais. J Infect Dis. 2009; 199:1442–8. [PubMed: 19392621]
28. Randall LM, Kenangalem E, Lampah DA, Tjitra E, Mwaikambo ED, Handojo T, Piera KA, Zhao ZZ, de Labastida Rivera F, Zhou Y, McSweeney KM, Le L, et al. A study of the TNF/LTA/LTB locus and susceptibility to severe malaria in highland papuan children and adults. Malar J. 2010; 9:302. [PubMed: 21029472]
29. Morales-Garcia G, Falfan-Valencia R, Garcia-Ramirez RA, Camarena A, Ramirez-Venegas A, Castillejos-Lopez M, Perez-Rodriguez M, Gonzalez-Bonilla C, Grajales-Muniz C, Borja-Aburto V, Mejia-Arangure JM. Pandemic influenza A/H1N1 virus infection and TNF, LTA, IL1B, IL6, IL8, and CCL polymorphisms in Mexican population: a case-control study. BMC Infect Dis. 2012; 12:299. [PubMed: 23148654]
30. Azevedo ZM, Moore DB, Lima FC, Cardoso CC, Bougleux R, Matos GI, Luz RA, Xavier-Elsas P, Sampaio EP, Gaspar-Elsas MI, Moraes MO. Tumor necrosis factor (TNF) and lymphotoxin-alpha (LTA) single nucleotide polymorphisms: Importance in ARDS in septic pediatric critically ill patients. Human Immunology. 2012; 73:661–7. [PubMed: 22507624]
31. Seidemann K, Zimmermann M, Book M, Meyer U, Burkhardt B, Welte K, Reiter A, Stanulla M. Tumor necrosis factor and lymphotoxin alfa genetic polymorphisms and outcome in pediatric patients with non-Hodgkin's lymphoma: results from Berlin-Frankfurt-Munster Trial NHL-BFM 95. J Clin Oncol. 2005; 23:8414–21. [PubMed: 16293872]
32. Ibrahim A, Abdel Rahman H, Khorshied M, Sami R, Nasr N, Khorshid O. Tumor necrosis factor alpha-308 and Lymphotoxin alpha+252 genetic polymorphisms and the susceptibility to non-Hodgkin lymphoma in Egypt. Leuk Res. 2012; 36:694–8. [PubMed: 22177457]
33. Wang SS, Bratti MC, Rodriguez AC, Herrero R, Burk RD, Porras C, Gonzalez P, Sherman ME, Wacholder S, Lan ZE, Schiffman M, Chanock SJ, et al. Common variants in immune and DNA repair genes and risk for human papillomavirus persistence and progression to cervical cancer. J Infect Dis. 2009; 199:20–30. [PubMed: 19012493]
34. Gallicchio L, Chang H, Christo DK, Thuita L, Huang H-Y, Strickland P, Ruczinski I, Hoffman SC, Helzlsouer KJ. Single nucleotide polymorphisms in inflammation-related genes and mortality in a community-based cohort in Washington county, Maryland. American Journal of Epidemiology. 2008; 167:807–13. [PubMed: 18263601]
35. Oh SS, Chang SC, Cai L, Cordon-Cardo C, Ding BG, Greenland S, He N, Jiang Q, Kheifets L, Le A, Lee YC, Liu S, et al. Single nucleotide polymorphisms of 8 inflammation-related genes and their associations with smoking-related cancers. Int J Cancer. 2010; 127:2169–82. [PubMed: 20112337]
36. Ovsyannikova IG, Vierkant RA, Pankratz VS, Jacobson RM, Poland GA. Extended LTA, TNF, LST1 and HLA gene haplotypes and their association with rubella vaccine-induced immunity. PLoS One. 2010; 5:e11806. [PubMed: 20668555]

Research novelty

The toll-like receptor and the nuclear factor κB signaling pathways are thought to be involved in response to the human papillomavirus infection. Here, the authors investigate whether genetic variation in these pathways may confer susceptibility to cervical and vulvar cancers. They found that the TNF-region was significantly associated with both cancers. A single nucleotide polymorphism in this region was found to significantly increase cervical cancer risk by 31% and vulvar cancer risk by 51%.

Table 1

Gene-based association analysis for cervical and vulvar cancers, Seattle-Puget Sound Region, 1986–2004.

			Cervical cancer			Vulvar cancer		
Gene region	**Chr.**	**Number of SNPs within gene region**	**All cases P-value**	**SCC P-value**	**Adenocarcinoma P-value**	**All cases P-value**	**HPV+ P-value**	**HPV- P-value**
TNFRSF1B	1	18	0.967	0.797	0.956	0.144	0.285	**0.018**
IKBKE	1	19	**0.021**	0.143	**0.038**	0.983	0.950	0.314
TRAF2	2	6	0.281	0.531	0.220	0.077	0.047	0.697
AZI2	3	4	0.315	0.531	0.503	0.664	0.885	0.586
TLR9	3	2	0.223	0.306	0.101	0.116	0.218	**0.031**
NFKB1	4	14	0.912	0.925	0.559	0.469	0.643	0.241
TLR3	4	11	0.412	0.660	0.524	0.512	0.746	0.777
MAP3K1	5	1	0.148	0.532	0.112	0.823	0.850	0.756
TICAM2	5	7	0.906	0.689	0.963	0.441	0.695	0.224
TNF-region	6	12	**2.0×10^{-4}**	**1.5×10^{-3}**	**0.013**	**1.0×10^{-4}**	**1.0×10^{-4}**	0.506
MAP3K7	6	6	0.363	0.548	0.034	0.986	0.723	0.908
TLR4	9	12	0.905	0.859	0.712	0.418	0.546	0.653
NFKB2	10	3	**0.005**	**0.034**	**0.045**	0.340	0.817	0.106
TOLLIP	11	17	0.337	0.229	0.609	0.515	0.909	0.211
TRAF6	11	4	0.379	0.079	0.712	0.952	0.919	0.439
RELA	11	3	0.358	0.257	0.590	**0.011**	**0.003**	0.590
TIRAP	11	8	0.199	0.743	0.238	**2.6×10^{-3}**	**0.040**	0.378
TNFRSF1A	12	5	0.655	0.170	0.982	0.550	0.507	0.052
IRAK4	12	4	0.981	0.993	0.919	0.262	0.290	0.771
TBK1	12	5	0.238	0.526	0.216	0.994	0.641	0.198
TRAF3	14	12	0.717	0.915	0.346	0.245	0.246	0.923
TICAM1	19	4	0.759	0.484	0.189	0.887	0.787	0.530
RELB	19	4	0.860	0.547	0.300	0.655	0.413	0.116
IRF3	19	3	0.833	0.284	0.303	0.109	0.402	0.096
VISA	20	7	0.823	0.544	0.739	0.852	0.641	0.917
ZBP1	20	7	0.184	0.490	0.175	0.549	0.083	0.506
TLR7	23	7	0.561	0.538	0.241	**0.007**	**0.004**	0.113

Number of controls: 1,100; number of cervical cases: 886; number of vulvar cases: 517. Bold indicates significant at the 5% level. P-values less than $0.05/27 \times 1.9 \times 10^{-3}$ were statistically significant after Bonferroni adjustment for multiple testing.

Table 2

Association between tagSNPs in the TNF region and cervical cancer risk, Seattle-Puget Sound Region, 1986–2004.

		All cervical cancer				Cervical SCC				Cervical adenocarinoma			
SNPs	**MAF Controls (N=1,100) (%)**	**MAF Cases (N=876) (%)**	**OR***	**(95% CI) ***	**Adj. P‡**	**MAF Cases (N=403) (%)**	**OR***	**(95% CI) ***	**Adj. P‡**	**MAF Cases (N=473) (%)**	**OR***	**(95% CI) ***	**Adj. P‡**
rs2009658	17.12	13.00	0.72	(0.59–0.86)	0.091	11.75	0.64	(0.5–0.82)	0.101	14.03	0.79	(0.63–0.99)	1.000
rs915654	36.25	30.68	0.79	(0.69–0.91)	0.157	31.41	0.81	(0.68–0.97)	1.000	30.09	0.77	(0.65–0.92)	0.608
rs2239704	37.65	43.80	1.31	(1.15–1.50)	**0.013**	43.98	1.31	(1.11–1.55)	0.342	43.66	1.31	(1.11–1.54)	0.298
rs2229094	27.92	22.38	0.72	(0.62–0.84)	**0.005**	20.94	0.67	(0.55–0.82)	**0.020**	23.55	0.77	(0.64–0.93)	1.000
rs2229092	6.95	5.89	0.83	(0.63–1.09)	1.000	5.11	0.71	(0.49–1.03)	1.000	6.53	0.95	(0.69–1.32)	1.000
rs1799964	23.08	18.18	0.72	(0.61–0.85)	**0.016**	16.71	0.66	(0.53–0.82)	**0.036**	19.38	0.77	(0.63–0.94)	1.000
rs1800610	7.53	9.29	1.24	(0.99–1.55)	1.000	9.53	1.27	(0.96–1.69)	1.000	9.10	1.22	(0.93–1.6)	1.000
rs3093662	7.95	6.41	0.74	(0.57–0.96)	1.000	5.87	0.69	(0.49–0.98)	1.000	6.85	0.78	(0.57–1.07)	1.000
rs769178	7.26	9.19	1.27	(1.01–1.60)	1.000	9.37	1.29	(0.97–1.73)	1.000	9.05	1.26	(0.96–1.66)	1.000
rs2256965	40.26	44.59	1.20	(1.06–1.37)	1.000	45.17	1.22	(1.04–1.44)	1.000	44.11	1.19	(1.01–1.39)	1.000
rs1052248	29.45	25.38	0.80	(0.69–0.92)	0.476	23.82	0.74	(0.61–0.9)	0.445	26.66	0.84	(0.71–1.01)	1.000
rs11575839	2.47	1.66	0.67	(0.41–1.09)	1.000	1.66	0.66	(0.35–1.26)	1.000	1.67	0.69	(0.38–1.25)	1.000

MAF: Minor allele frequency.

*Age-adjusted OR assuming an additive model.

‡P value adjusted for multiple testing using the Bonferroni adjustment across all 205 SNPs. Bold indicates significant at the adjusted 5% level.

Table 3

Association between tagSNPs in the TNF region and vulvar cancer risk, Seattle-Puget Sound Region, 1986–2004.

		All vulvar cancer				Vulvar cancer (HPV +)				Vulvar cancer (HPV -)			
SNPs	**MAF Controls (N=1,100) (%)**	**MAF Cases (N=517) (%)**	**OR***	**(95% CI) ***	**Adj. P‡**	**MAF Cases (N=403) (%)**	**OR***	**(95% CI) ***	**Adj. P‡**	**MAF Cases (N=473) (%)**	**OR***	**(95% CI) ***	**Adj. P‡**
rs2009658	17.12	13.09	0.72	(0.58–0.90)	0.652	12.79	0.70	(0.55–0.90)	1.000	16.67	0.93	(0.60–1.45)	1.000
rs915654	36.25	28.38	0.70	(0.59–0.82)	**3.2×10^{-3}**	27.09	0.65	(0.54–0.79)	**2.2×10^{-3}**	33.33	0.87	(0.61–1.22)	1.000
rs2239704	37.65	47.95	1.51	(1.30–1.75)	**1.9×10^{-5}**	49.00	1.58	(1.33–1.87)	**5.1×10^{-5}**	42.95	1.24	(0.89–1.73)	1.000
rs2229094	27.92	22.32	0.74	(0.62–0.88)	0.172	21.63	0.71	(0.58–0.87)	0.216	26.92	0.96	(0.66–1.39)	1.000
rs2229092	6.95	5.31	0.75	(0.54–1.03)	1.000	5.86	0.83	(0.58–1.19)	1.000	5.77	0.81	(0.41–1.62)	1.000
rs1799964	23.08	18.71	0.76	(0.63–0.92)	1.000	18.77	0.77	(0.62–0.96)	1.000	20.51	0.86	(0.57–1.29)	1.000
rs1800610	7.53	10.53	1.44	(1.12–1.86)	0.974	10.17	1.38	(1.04–1.85)	1.000	8.97	1.22	(0.70–2.13)	1.000
rs3093662	7.95	7.02	0.88	(0.66–1.18)	1.000	7.16	0.90	(0.64–1.26)	1.000	5.77	0.76	(0.38–1.54)	1.000
rs769178	7.26	10.72	1.53	(1.18–1.97)	0.233	10.46	1.48	(1.11–1.97)	1.000	8.97	1.27	(0.73–2.20)	1.000
rs2256965	40.26	49.41	1.43	(1.23–1.66)	**5.5×10^{-4}**	50.29	1.48	(1.25–1.76)	**1.4×10^{-3}**	47.44	1.32	(0.95–1.83)	1.000
rs1052248	29.45	24.07	0.76	(0.64–0.91)	0.414	23.35	0.73	(0.60–0.89)	0.465	27.56	0.92	(0.64–1.33)	1.000
rs11575839	2.47	2.33	0.95	(0.57–1.57)	1.000	2.69	1.09	(0.63–1.90)	1.000	0.65	0.25	(0.03–1.86)	1.000

MAF: Minor allele frequency.

*Age-adjusted OR assuming an additive model.

‡P value adjusted for multiple testing using the Bonferroni adjustment across all 205 SNPs. Bold indicates significant at the adjusted 5% level.