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Polymorphisms in immune mediators associate with risk of cervical cancer

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HIGHLIGHTS

- Family-based association is studied to identify susceptibility variants in immune response genes for cervical cancer.
- SNPs in *JAK2* and *STAT6* genes are identified to be significantly associated with the risk of cervical cancer.

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ABSTRACT

Objective. The immune system is critical for controlling the progression of HPV cervical disease and the development of cancer. This study aimed to identify cervical cancer susceptibility alleles in candidate immune-modulating genes.

Methods. Our family-based study involved a cohort of 641 probands (women with ICC/CIN III) and their biologic parents or siblings (641 trios). In the discovery phase (stage 1), involving 288 of the trios, 81 tag single nucleotide polymorphisms (SNPs) in 11 immune-modulating genes (*IFNG*, *IFNGR1*, *IFNGR2*, *JAK1*, *JAK2*, *STAT1*, *STAT6*, *IL12A*, *TNF*, *LTA* and *LTB*) were evaluated on the GoldenGate platform. We used the combined dataset for a total of 641 trios (stage 2) and the Taqman platform to validate the SNPs that had proved significant in the discovery dataset. The transmission disequilibrium test was used to detect significant shifts in allelic transmissions in the datasets.

Results. Two SNPs in *JAK2* and one SNP in *STAT6* showed significant allelic association with cervical cancer in the stage 1 discovery dataset and were replicated in the larger joint analysis stage 2 dataset (*JAK2* rs10815144, $P = 0.0029$ and rs12349785, $P = 0.0058$; and *STAT6* rs3024971, $P = 0.0127$). An additional SNP in exon 19 of *JAK2* (rs2230724) was also examined in the combined dataset due to its strong linkage disequilibrium (LD) with rs10815144. It was also significant ($P = 0.0335$).

Conclusions. Our results suggest an association of SNPs in *JAK2* and *STAT6* with cervical cancer. This association should be investigated in additional cervical cancer populations.

Introduction

Persistent high-risk HPV infection is essential for the development of cervical cancer [1]. However, 50%–90% of early cervical intraepithelial neoplasia (CIN) cases regress spontaneously [2,3], suggesting a genetic influence. Previous evidence for host genetic factors contributing to susceptibility to cervical intraepithelial neoplasia (CIN) and invasive cervical cancer (ICC) has come from family-based and case–control studies [4–8].

Both clinical observations and experimental research suggest that the host immune system plays a critical role in controlling HPV infections. For example, immunosuppressed women have increased incidence of HPV infections, CIN lesions, and prolonged persistence of intraepithelial lesions [9,10]. Clearance or persistence of HPV infection is dependent on local cell-medicated immunity. Stromal dendritic cells expressing immunosuppressive factors were more numerous in stroma of cancerous cervical biopsies than in normal cervix [3]. An imbalance of local inflammatory cytokines, such as TNF-alpha, interferon (IFN)-gamma, and interleukin 12 (IL-12), associates with persistent HPV infection and disease progression [3,11,12].

IL-12 is a pro-inflammatory cytokine that triggers the production of IFN-gamma and regulates many cellular functions, including anti-viral and tumor immune-surveillance [13]. Several studies have examined polymorphisms in IL-12 subunits and risk of cervical cancer, but with variable results [14,15]. INF-gamma is involved in both innate and adaptive immunities, and a defect in peripheral blood lymphocyte

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IFN-gamma signaling is found when cancer patients are compared to healthy controls [16]. The major signaling pathway activated by IFN-gamma involves sequential phosphorylation of the tyrosine residues of the Janus kinases (JAK-1 and JAK-2) and then the signal transducer and activator of transcription (STAT) proteins, providing the primary mechanism for gene induction [13]. Germ-line variants and somatic mutations in genes of the JAK-STAT pathway associate with a variety of cancers, including breast cancer, prostate cancer, and leukemia [17–19].

Tumor necrosis factor (*TNF*), lymphotoxin alpha (*LTA*), and lymphotoxin beta (*LTB*) genes are members of the tumor necrosis factor superfamily. They cluster within the chromosomal 6p21.3 region. TNF is a cytokine involved in systemic inflammation, apoptosis, tumorigenesis, and viral replication [20]. LTA is a pro-inflammatory cytokine with anti-tumor activity and is in linkage disequilibrium with the *TNF* promoter. Upregulation of TNF and other inflammatory cytokines in keratinocytes after viral infection is significantly reduced in HPV-positive keratinocytes. The altered levels of TNF may influence the immune response to pathogens and contribute to an individual's susceptibility to cancer [1, 20]. Polymorphisms in the *TNF* promoter and *LTA* genes have been shown to associate with susceptibility to cervical cancer [20–22].

In this study, we investigated 81 tag single nucleotide polymorphisms (SNPs) in 11 immune response genes involved in the IFN-gamma signaling pathway and a cluster of TNF super-family genes. Our hypothesis was that common polymorphisms influencing immune-modulating genes may affect the development of cervical cancer, because the balance between HPV's immune evasion and eradication by a host's immune system significantly determines the development of the disease.

Materials and method

Study population

A total of 641 subjects with CIN3 (CIN3 and/or adenocarcinoma in situ) or ICC and their biological parents or one parent and one or more siblings participated in the study. DNA was extracted from blood or buccal cells from all participants as previously reported [7]. Samples used in this study were collected under a protocol approved by the Human Research Protection Office (HRPO) at Washington University in St. Louis and the Human Research Protection Program at Medical College of Wisconsin. HPV was typed as described previously [7]. Families were grouped according to the HPV type detected in the probands' cervical neoplasia at diagnosis. HPV16-related types included HPV16, HPV31, and HPV52. HPV18-related types included HPV18 and HPV45. The subjects were infected with only one of those types or with several of those plus other types of HPV. Characteristics of subjects and cervical tumors are provided in Table 1.

Table 1
Subject characteristics.

	Subjects
Total	641
Race	
Caucasian	572
African American	66
Asian American	3
Age	
Mean age (y) ± SD	34.39 ± 8.77
Stage	
0 (CIN3, adenocarcinoma in situ)	253
I	317
II	47
III	17
IV	2
Unstaged	5
Histology for invasive cancers	
Squamous cell	244
Adenocarcinoma	104
Adenosquamous	16
Others	19
Unknown	5

Candidate genes and tag SNP identification

We included 81 SNPs from 11 candidate genes (*IFNG*, *IFNGR1*, *IFNGR2*, *JAK1*, *JAK2*, *STAT1*, *STAT6*, *IL12A*, *LTA*, *TNFα* and *LTB*; Supplemental Table 1) on the GoldenGate array (Illumina, Inc., San Diego, CA). Haploblocks representing tag SNPs were identified for these genes, using the tagger program at http://www.broad.mit.edu/mpg/tagger/. We screened 5 kb both upstream and downstream of the candidate gene in the genomic region, and we captured LTA when we designed SNPs in the 5′ end of TNF.

Genotyping

The GoldenGate assay is very sensitive to DNA quantity and quality. Therefore, we quantified the percentage of functional template in each blood and buccal DNA sample, using a standardized TaqMan RNase P Detection kit (Applied Biosystems, Foster City. CA). The 5′ nuclease assay quantifies genomic copies of the single-copy human RNase P gene [23]. DNAs of 288 trios (discovery dataset) were genotyped by the Illumina GoldenGate genotyping platform (Illumina Inc., San Diego, CA). Genomic DNA (250 ng to 750 ng per sample) was used for each assay. The genotyping was done by the Genome Technology Access Center (GTAC) at Washington University in St. Louis. DNAs of the entire 641 family trios (stage 2 joint analysis dataset) were genotyped by the TaqMan Genotyping Assay for SNPs rs10815144, rs12349785, and rs2230724 in *JAK2* and rs3024971 in *STAT6*. This workflow allowed us to identify genotyping errors between platforms and Mendelian errors in families and to assess statistical significance in a larger stage 2 joint analysis set.

Statistical methods

We used the family-based transmission disequilibrium test (TDT) implemented in the program TRANSMIT as previously described [5,24, 25]. In the TDT, an association between a SNP and cervical cancer is tested by comparing the number of alleles transmitted from heterozygous parents to an affected daughter with the number of nontransmitted alleles. Deviation from the 1:1 ratio suggests an association between an allele and cervical cancer risk. The final genotypes were analyzed for transmission consistency between parents and offspring, and genotypes that showed Mendelian errors were excluded from analysis.

A two-stage design was used to evaluate genetic variation. First, 81 SNPs in the discovery set of 288 trios were screened. SNPs significant at a nominal $P = 0.05$ were then genotyped in a stage 2 joint analysis of all samples yielding a total of 641 trios [26]. To identify possible heterogeneity of risk, we subdivided the trios by race, stage, and HPV type, as in previous studies [5,7]. Subjects with tumors positive for 16- and 18-related HPVs were compared with subjects whose tumors contained strains of HPV other than 16- and 18-related.

Results

Eighty-one tag SNPs from 11 immune-modulating genes were evaluated in a two-stage family-based association study. All SNPs were genotyped in more than 99% of the samples. The Mendelian error rate was <5%, and the inconsistent genotypes were excluded from analysis, after verifying the reported parent–daughter relationships. Overall, 3 polymorphisms in 2 genes (*JAK2*, *STAT6*) were found to be significantly ($P < 0.05$) associated with cervical cancer in the 288 discovery trios (Table 2). To replicate the associations, the significant

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