

## BRIEF COMMUNICATION

### Human Papillomavirus Type 16 Variants and Risk of Cervical Cancer

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There are substantial data demonstrating that human papillomaviruses (HPVs) are the sexually transmitted etiologic agents of cervical cancer (1). HPV type 16 (HPV16) is the most common HPV type detected in tumors, accounting for 50% of cancers and their precursors, called high-grade squamous intraepithelial lesions (HSILs) (2). Preliminary studies (3–16) have suggested that variants of HPV16 may show varying degrees of association with cervical neoplasia. This may partially explain why some HPV16 infections progress to HSIL or cancer, while others do not. If causal, these associations may be explained by differences in the transcriptional regulation of the virus by different variants, in the biologic activities of the proteins encoded by HPV16 variants (e.g., enhanced transforming abilities of E6/E7), or in the ability of the host to respond immunologically to specific viral epitopes encoded by variants. This last effect is likely to be mediated through human leukocyte antigen (HLA) presentation of viral antigens (17–21). Previous studies of viral variants and cervical neoplasia have relied on convenience samples, limiting their interpretation, while others have had small sample size.

We conducted a prevalent case-control study within a 10 000-woman, population-based cohort in Costa Rica to examine the association between

HPV16 variants and cervical neoplasia. In addition to its size and population-based design, our study has the advantage of having been conducted in a highly admixed population. Our investigation was approved by institutional review boards in the United States and in Costa Rica. All participants provided written informed consent. The cohort from which this study derives has been described (22–24) and consists of 10 077 women (>93% response rate) who were interviewed and screened for cervical abnormalities by use of conventional cytology and newer screening methods (ThinPrep, Papnet, and cervicography). The cohort consists of 10 049 women randomly selected from the general population and an additional 28 women (22) from the same ascertainment area diagnosed with cancer and representing 90% of all cervical cancer cases diagnosed among women from Guanacaste, Costa Rica, during the cohort ascertainment period. Those women with an abnormal screening test were referred to colposcopy, where biopsy specimens were taken. Review of cytohistologic data revealed 40 cancers (12 within our random sample plus the 28 cancer cases described above), 128 HSILs, 189 low-grade squamous intraepithelial lesions (LSILs), 661 equivocal lesions, and 7564 cytologically normal results (22,23,25). The entire cohort was screened for HPV DNA by the use of the Hybrid Capture Tube test (Digene Corp., Gaithersburg, MD) (24,26). Polymerase chain reaction (PCR)-based testing was also performed on more than 40% of cohort members, including all women with any screening abnormality, those who were positive by hybrid capture, and a sample of the remaining cohort (23). HPV16 DNA by PCR was detected in 190 subjects. Sequencing of the long control region (nucleotides 7408–7891) of HPV16, which contains the highest degree of variation in the viral genome, was performed in a blinded fashion on 176 subjects (16 cancers, 56 HSILs, 20 LSILs, and 84 with equivocal lesions or normal diagnosis).<sup>1</sup> PCR products were confirmed by agarose gel electrophoresis and purified by use of the Quickstep PCR kit (Edge BioSystems, Gaithersburg, MD). Sequences were determined by cycle sequencing.

Host genotyping was also performed to determine the degree of genetic relatedness of individuals in our study with

the use of a set of commercially available microsatellite markers (AmpFISTR™ ProfilerPlus; PE Corporation, Foster City, CA) (27). Specimens were tested by the use of the kit reagents and a modified protocol (28). Genotyping was performed on a subset of 140 women, including all 16 cancers, 55 HSILs, and 69 women with LSIL, equivocal lesions, or normal cytology, and was successful for all specimens but two tested. The genetic distance between groups was computed by Nei's method (29) to determine whether subjects with prototype and variant forms of HPV16 were genetically heterogeneous (29) so that we could address concerns of population stratification arising from the coevolution of HPV with the human species. At the time of this analysis, high-resolution HLA class II DRB1 and DQB1 testing was available for 95 and 98, respectively, of the 176 study participants from a parallel study within our cohort (manuscript in preparation). HLA testing was performed by a PCR-based sequence-specific oligonucleotide probe method as described previously (30–32).

The European-derived HPV16 prototype virus (EP[g]) was detected in 36 subjects. In addition, three distinct variant groups were observed. The most common variant (EP[a]) was a G to A substitution at position 7521. In contrast to EP[g], EP[a] was not associated with disease (relative risk [RR] for HSIL = 1.0; RR for cancer = 1.3; overall  $P = .94$ ) and was combined with EP[g] for analysis. Twenty subjects were found to contain variants that, other than at posi-

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**Table 1.** Percent distribution of human papillomavirus (HPV) 16 LCR variants by degree of cervical neoplasia in the Costa Rica study\*,†

Variant	Control subjects,‡ % distribution§	Disease status			
		HSIL		Cancer	
		% distribution§	RR (95% CI)	% distribution§	RR (95% CI)
European prototype	80.7%	75.0%	1.0 (referent)	56.3%	1.0 (referent)
European-like	13.5%	10.7%	0.86 (0.25–2.6)	0%	0 (0.0 to 3.5)
Non-European	5.8%	14.3%	2.7 (0.75–9.9)	43.7%	11 (2.5 to 50)
Total	104 (100%)	56 (100%)		16 (100%)	

\*LCR = long control region; HSIL = high-grade squamous intraepithelial lesion; RR = relative risk; CI = confidence interval.

†Exact Pearson *P* value (two-sided) <.001 (calculated by use of StatXact; Cytel Software Corp., Cambridge, MA).

‡Defined as HPV16-positive women with low-grade squamous intraepithelial lesions, equivocal lesions, or normal cytology.

§Percent distribution.

||RR and 95% CI estimates compared with control subjects. Estimates derived by use of exact methods (33).

tion 7521, varied from EP at a single nucleotide position (European-like [EL] variants). The remaining 21 subjects had substitutions at multiple positions, most notably at positions 7485, 7489, 7669, 7689, 7729, 7764, 7786, and 7886 (non-EL [NE] variants). Nucleotide sequences observed in our study can be found on the *Journal of the National Cancer Institute* website (<http://jnci.oupjournals.org>).

We reported previously that women positive for HPV16 in our cohort were 320 (95% confidence interval [CI] = 97 to 1000) and 710 (95% CI = 110 to 4500) times more likely than HPV-negative women to be diagnosed with HSIL and cancer, respectively (23). In the present study, we evaluated whether, among HPV16-positive individuals, those infected with specific variants are at an even higher risk of disease. We compared HPV16-positive cancer and HSIL cases against a control group consisting of HPV16-positive women without evidence of HSIL or cancer (i.e., women with LSIL, equivocal lesions, or normal cytology). A striking association was observed between variant groups and disease (two-sided *P*<.001; Table 1). NE variants were seen in 5.8% of control subjects but in 14.3% of HSILs and in 43.7% of cancers. Conversely, EL variants were detected in 13.5%, 10.7%, and 0% of control subjects, HSILs, and cancers, respectively.

Women with HSIL or cancer who tested positive for the NE variant had an RR of 2.7 (95% CI = 0.75 to 9.9) and 11 (95% CI = 2.5 to 50), respectively. No statistically significant association was observed for individuals positive for the EL variant (95% CIs overlapped 1.0). Adjustment for age did not mate-

rially affect estimates. Previous studies (3,4) have also suggested that those with NE variants were associated with anogenital cancers.

If NE variants are more aggressive, one might expect HSILs and cancers associated with NE variants to occur at earlier ages than those associated with EP/EL variants. However, no differences in the diagnosed age of HSIL or cancer in patients were noted between those positive for NE versus EP/EL variants. The median age of patients diagnosed with cancers with NE variants was 41 years compared with 34 years for those diagnosed with EP/EL variants (mean age = 45 and 43.2 years, respectively; *P* = .81). The median age of

diagnosis of HSIL cases with NE variants was 32.5 years compared with 34.5 years for HSIL cases with EP/EL variants (mean age = 35 and 38.1 years, respectively; *P* = .56). For comparison, the median age of our control subjects was 32 years (mean = 37.1 years).

We examined whether our findings were due to cosegregation of variants among subpopulations (defined by geography, socioeconomic status, or genetic relatedness), with potentially different risks of cervical neoplasia. No notable differences were observed in the distribution of these factors when women infected with the NE and EP/EL variants were compared (Table 2). When genetic relatedness was exam-

**Table 2.** Percent distribution of sociodemographic factors by human papillomavirus (HPV) 16 LCR variant group in the Costa Rica study\*

Factor	NE (n = 21)	EL/EP (n = 155)	<i>P</i> †
Education			.23
None	4.8%	11.0%	
Some primary	47.6%	22.5%	
Completed primary	28.6%	28.4%	
Secondary/vocational	9.5%	27.1%	
College‡	9.5%	11.0%	
Geography, by location			.74
North	38.1%	29.7%	
South	23.8%	18.7%	
West	23.8%	30.3%	
East	14.3%	21.3%	
Geography, by prevalence‡			.68
<1.2%	19.1%	19.3%	
1.2–<2.0%	23.8%	27.1%	
2.0–<2.5%	19.1%	23.9%	
≥2.5%	38.1%	29.7%	

\*LCR = long control region; HSIL = high-grade squamous intraepithelial lesion; NE = non-European variant; EL = European-like variant; EP = European prototype variant.

†Calculated by use of exact methods (calculated by use of StatXact; Cytel Software Corp., Cambridge, MA). *P* values presented are the Pearson *P* value for geography (by location) and the trend *P* value for education and geography (by prevalence).

‡Guanacaste regions (Cantones) were subdivided based on the prevalence of HSIL or cancer observed during the enrollment phase of our 10 077-woman cohort study.

ined, we observed no evidence of genetic stratification between those with the NE variant and those with the EP/EL variant. Nei's genetic distance between women with and without the NE variant was less than .05 (on a scale from 0 to 1, with 0 indicating complete homogeneity between groups).

We also examined whether the distribution of HLA DRB1-DQB1 haplotypes differed among women with EP/EL and NE variants, since HLA is responsible for the presentation of viral antigens to the immune system and specific HLA haplotypes might be associated with inadequate immune presentation of epitopes encoded by HPV16 variants. Detection of NE variants was associated with the presence of HLA DRB1\*1102-DQB1\*0301 (two-sided *P* value for Fisher's exact test = .0005).

Our data provide epidemiologic evidence, from a population-based study, that different HPV16 variants have different oncogenic potential. Combined with our previous finding that HPV16 infection (all variants combined) in our Guanacaste population is associated with a 710-fold increased risk of cervical cancer, the present finding that those infected with NE variants of HPV16 are 11 times more likely than those infected with the prototype HPV16 virus to be diagnosed with cervical cancer suggests that the overall risk associated with infection with NE variants of HPV16 is indeed large.

The reported associations are unlikely to be due to cosegregation of variants with subpopulations at higher risk of disease, and the observed HLA associations suggest the possibility that differential immune handling of different variants may partially explain our findings. Whether the association is due to a direct effect of the LCR variants detected here or to an indirect effect resulting from linkage disequilibrium with variations present in other regions of the viral genome remains to be determined.

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## NOTES

<sup>1</sup>Sequencing was not performed for 11 subjects because of an oversight that resulted in their not being selected for study and for three subjects because of inadequate specimens.

*Editor's note:* M. E. Sherman is or has recently been involved in research sponsored by or under contract with Digene Corp., TriPath (Burlington, NC), Merck, Cytec Inc., and National Testing Laboratories, all of whom manufacture products related to screening or diagnosis of cervical cancer.

Supported by a series of Public Health Service (PHS) contracts from the National Cancer Institute (NCI), National Institutes of Health (NIH), Department of Health and Human Services (DHHS), and in part by PHS grant CA78527 (to R. D. Burk) from the NCI, NIH, DHHS.

We thank Dr. Mario Alfaro (Costa Rican Social Security Administration, San Jose, Costa Rica) and Dr. Martha Hutchinson (Womens and Infants' Hospital, Providence, RI) for their careful review of cytology specimens, Dr. Jorge Morales (Costa Rican Social Security Administration, Guanacaste, Costa Rica) for performing the colposcopy and biopsy of study participants, Dr. Mitchell Greenberg (OMNIA, Blue Bell, PA) for interpreting cervicography images collected from participants, and Dr. Attila T. Lorincz (Digene Corp., Silver Spring, MD) for HPV DNA testing by hybrid capture (Digene Corp., Silver Spring, MD). We ac-

knowledge the collaboration of Julie Buckland, Linda Saxon, and Kay Helgesen (IMS, Rockville, MD) for data management. We also thank Robert Tarone (NCI, Bethesda, MD) for his assistance in performing exact statistical tests and Deidra Kelly (The Johns Hopkins University, Baltimore, MD) and Dr. Laurie Mango (Neuromedical Systems, Suffern, NY) for their collaboration in the interpretation of cytologic specimens. Reagents and services were supplied or discounted by Cytec Inc. (Boxborough, MA), National Testing Laboratories (Fenton, MO), Utah Medical (Midvale, UT), and Neuromedical Systems (Suffern, NY). We offer special recognition for the excellent work of the study staff in Costa Rica, in particular, Fernando Cardenas, Manuel Barrantes, Elmer Perez, Lidia Ana Morera, and Iris Ugarte. We also acknowledge the collaboration of health authorities in Costa Rica for their enthusiastic support of this project and of the outreach workers of the Ministry of Health of Costa Rica who carried out the population census for their dedication to the health of the people of Guanacaste.

Manuscript received June 13, 2000; revised November 21, 2000; accepted December 6, 2000.