

Risk for High-Grade Cervical Intraepithelial Neoplasia Associated with Variants of Human Papillomavirus Types 16 and 18

Long Fu Xi,^{1,2} Laura A. Koutsky,² Allan Hildesheim,⁴ Denise A. Galloway,³ Cosette M. Wheeler,⁵ Rachel L. Winer,² Jesse Ho,¹ and Nancy B. Kiviat¹

¹Department of Pathology, School of Medicine and ²Department of Epidemiology, School of Public Health and Community Medicine, University of Washington; ³Fred Hutchinson Cancer Research Center, Seattle, Washington; ⁴Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, Maryland; and ⁵Departments of Microbiology and Molecular Genetics, School of Medicine, University of New Mexico, Albuquerque, New Mexico

Abstract

Background: Although the variant lineages of human papillomavirus (HPV) types 16 and 18 are well established, their individual associations with high-grade cervical intraepithelial neoplasia (CIN) have not been extensively evaluated.

Methods: Study subjects were women participating in the Atypical Squamous Cells of Undetermined Significance/Low-Grade Squamous Intraepithelial Lesion Triage Study who were positive for HPV16 or HPV18 at enrollment. These women were followed every 6 months for 2 years. Viral isolates from enrollment samples were characterized by DNA sequencing and classified as variant lineages.

Results: Over a 2-year study period, CIN3 was histologically diagnosed in 291 of the 779 HPV16-positive women and 47 of the 275 HPV18-positive women. Among women without CIN2-3 at enrollment, the risk of subsequent CIN3 was 2.7-fold greater for those with HPV16 African-2 [95% confidence

interval (95% CI, 1.0-7.0)] and 3.1-fold greater for those with HPV16 Asian American (95% CI, 1.6-6.0), compared with European variants. Relative to infection with HPV18 African variants, the risk associating subsequent CIN3 was 3.8 (95% CI, 0.9-17.2) for infection with HPV18 European variants and 4.8 (95% CI, 1.0-23.6) for infection with HPV18 Asian American variants. Similar associations were observed when the 2-year prevalence of CIN3 was used as the end point. Further, for those with HPV16 European variants, the 2-year prevalence of CIN3 was higher in White women than in African American women ($P = 0.01$); this trend was reversed for those with HPV16 African-1 variants ($P = 0.22$). A similar pattern was present for infections with HPV18 European versus African variants.

Conclusions: The lineages of HPV16 and HPV18 variants are associated with differing risks for high-grade CIN. (Cancer Epidemiol Biomarkers Prev 2007;16(1):4-10)

Introduction

There are substantial data (1-4) showing a variety of natural variants for any given type of human papillomavirus (HPV). These variants are generally classified and named according to their geographic relatedness (5-8). Interest in HPV variants is growing rapidly, as increasing evidence suggests that HPV variants may differ biologically and etiologically (9).

Thus far, studies on clinical relevance have focused mainly on the variants of HPV16, the type that confers the highest risk of cervical cancer (10) and also the type most commonly detected in women with normal cervical cytology (11). A classification of the variants on the basis of the presence of a single nucleotide alteration usually yielded inconsistent findings (12-23). Although data from studies comparing European (prototype-like) with non-European (non-prototype-like) variants have been relatively consistent, with non-European variants being associated with an increased risk of cervical lesions (24-28), a lack of association has also been reported (29-31). The majority of these studies, however, were cross-sectional in design, with a limited ability to adequately capture the variant-related outcomes of interest. Although a few

longitudinal studies have been reported (23-26, 29), the findings were limited by small sample sizes. Moreover, in many instances, it was not possible to distinguish the risk differences between the non-European lineages.

Much less is known about the clinical relevance of the variants of HPV18, although it is the second most common HPV infection detected in cervical cancer specimens (10) and is the type most strongly associated with adenocarcinoma of the cervix (31-33). Limited data suggest that certain HPV18 variants are more likely to be detected in adenocarcinomas and others in squamous cell carcinomas (34-36). In addition, an unbalanced variant distribution, although not statistically significant, was noted between women with and without cervical cancer (37). A recent cohort study (25) showed a tendency for an increased risk of high-grade cytologic abnormalities associated with non-European, compared with European, variants. However, it was not possible to dissect the independent role of HPV18 variants because the risks were assessed for either the grouped HPV16 and HPV18 variants or the HPV16 variants alone.

Data from a recent study have shown a race-associated difference in persistence of HPV16 and HPV18 variants, with European variants more likely to persist in White women and African variants more likely to persist in African American women (38). Because persistent infection with oncogenic HPV types is a significant biomarker for cervical diseases (39-43), a potential effect of race on the variant-related risk of cervical lesions deserves consideration.

In the present study, we examined the associations of risks of high-grade cervical intraepithelial neoplasia (CIN) with individual lineages of HPV16 and HPV18 variants in a large

Received 8/8/06; revised 10/19/06; accepted 11/8/06.

Grant support: USPHS grant CA 84396 (L.F. Xi).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Note: This study was part of the project ancillary to the ALTS clinical trial but does not represent the ALTS Group.

Requests for reprints: Long Fu Xi, University of Washington, 1914 North 34th Street, Suite 300, Seattle WA 98103. Phone: 206-616-9787; Fax: 206-616-9788. E-mail: longfu@u.washington.edu
Copyright © 2007 American Association for Cancer Research.

doi:10.1158/1055-9965.EPI-06-0670

number of women participating in the Atypical Squamous Cells of Undetermined Significance (ASCUS)/Low-Grade Squamous Intraepithelial Lesion (LSIL) Triage Study. By stratifying study subjects on race, we further evaluated effects of race on the variant-related disease risk.

Materials and Methods

Study Subjects. Study subjects were women who participated in the ASCUS/LSIL Triage Study (ALTS), a multicenter randomized clinical trial designed to evaluate strategies for triaging women with mildly abnormal Pap smears. Details on the design and study population of the ALTS trial have been described elsewhere (44, 45). Briefly, at enrollment, participants were randomly assigned into one of three management arms: immediate colposcopy, HPV triage, and conservative management. All of the participants underwent an entry procedure that included an interview, Pap smear, and collection of a cervical sample for HPV testing. Additionally, colposcopic examination with colposcopically directed biopsy of visible lesions was done for all women in the immediate colposcopy arm, those with high-risk HPV types in the HPV triage arm, and those with a cytologic diagnosis of high-grade squamous intraepithelial lesion (HSIL) in the conservative management arm. Regardless of the study arm, participants were followed at 6-month intervals for 2 years with cervical cytology and HPV testing. Women were re-referred for colposcopy and biopsy if cytologic evidence of HSIL was found during follow-up. At exit, participants were required to undergo an exit procedure, including cervical cytology, HPV testing, and colposcopic examination, with biopsy of any visible lesions. The institutional review board at National Cancer Institute and each of the four clinical sites involved in the trial approved the study protocol.

ALTS participants were eligible for the present study if they had HPV16 and/or HPV18 DNA detected by PCR-based reverse line strip assay in their enrollment cervical samples. In total, 1,114 women were identified, including 784 positive for HPV16 alone, 268 positive for HPV18 alone, and 62 positive for both. In the following analyses, a woman would be double counted if both HPV16 and HPV18 DNAs were detected in her cervical sample. We excluded 103 women [50 (5.9%) with HPV16 and 53 (16.1%) with HPV18] from the study because of failure to PCR-generate target fragments for DNA sequencing, leaving 796 HPV16-positive and 277 HPV18-positive women in analyses. Compared with women with HPV16 infections who were included in the study, those who were excluded were less likely to have a diagnosis of CIN3 and more likely to be ≥ 30 years of age, of African American descent, and currently using hormonal contraceptives; however, no substantial differences were observed with respect to lifetime number of sex partners, number of Pap tests in the past 5 years, referral cytology, or smoking status (data not shown). Among women with HPV18 infections, those who were excluded from the study did not differ significantly from those who remained in the study with respect to CIN3 occurrence, age at enrollment, self-reported race, use of hormonal contraceptives, lifetime number of sex partners, referral cytology, or number of annual Pap tests in the last 5 years (data not shown).

Clinical End Point. In ALTS, cervical cytology and histology were initially diagnosed by the clinical center pathologists and then reviewed by a panel of expert pathologists for quality control and safety monitoring. The main outcome of interest was first episode of CIN3 (unless otherwise specified) histologically confirmed by the panel of expert pathologists. For women with more than one diagnosis at a single visit, the most severe one was used as the final diagnosis.

Of the 796 women with HPV16 infections, 685 (86.1%) had at least one histologic diagnosis by the panel of expert patholo-

gists. Seventy-six of the 111 women without such a diagnosis had at least one histologic evaluation by the clinical center pathologists; all of them had normal histology. Cytologic diagnoses by the panel of expert pathologists were available for the remaining 35 women, including 7 with normal cytology, 11 with ASCUS, 12 with LSIL, and 5 with HSIL. Of the 277 women with HPV18 infection, 229 (82.7%) had at least one histologic diagnosis by the panel of expert pathologists. Thirty of the 48 women without such a diagnosis had at least one histologic evaluation by the clinical center pathologists; all of them had normal histology. Cytologic evaluation by the panel of expert pathologists was available for the remaining 18 women, including 5 with normal cytology, 2 with ASCUS, 9 with LSIL, and 2 with HSIL. None of the 7 women with HSIL (5 with HPV16 and 2 with HPV18) provided follow-up visits. In analyses of the risk association, these seven women were excluded and the remaining ones (106 with HPV16 and 46 with HPV18) were treated as not having CIN2-3.

Similar results were obtained when the diagnoses by the clinical center pathologists were used as the clinical end points. For simplicity, these results are not presented.

Characterization of HPV16 and HPV18 Variants. The protocol for characterization of HPV16/HPV18 variants by sequencing of PCR products has been described previously (38). Briefly, fragments of the target genes of HPV16 (751 bp, from nucleotide position 7,723-567) and HPV18 (956 bp, from nucleotide position 7,489-587), corresponding to the 3' part of the long control region and the entire E6 region, were PCR-generated, gel-isolated, and then purified with a QIAEX II gel extraction kit (Qiagen, Valencia, CA). Then, sequence variation was determined from both directions using a BigDye Terminator Cycle Sequencing kit according to the protocol of the manufacturer (Applied Biosystems, Foster City, CA). A viral isolate was defined as a distinct variant if one or more nucleotide alterations (relative to the prototype and other isolates) were detected in the region analyzed. According to the lineages categorized previously (5-8), HPV16 variants were classified as European, Asian, Asian American, African-1, African-2, and North American variants; HPV18 variants were classified as European, Asian American, and African variants.

Statistical Analyses. Unconditional logistic regression (46) was used to examine risk of the 2-year prevalence associated with HPV16 or HPV18 variants. The prevalent event was defined as histologically confirmed CIN3 (or \geq CIN2) detected at any time during the study period. For women with more than one CIN3 diagnosis, only the first episode was counted. The odds ratios (OR) with 95% confidence intervals (95% CI) associating risk of CIN3 with the variants were adjusted for study arm (the immediate colposcopy, HPV triage, or conservative management), self-reported race (White, African American, or American Indian/Alaskan/Asian/Pacific Islander), and age (18-19, 20-24, 25-29, or ≥ 30 years), current use of hormonal contraceptives (yes or no), and current smoking status (yes or no) at enrollment. A selection of these variables as covariates was based on their possible relations to variant exposure and disease outcome and on our previous findings of race-related variant distribution (38).

Among women without histologically confirmed CIN2-3 at enrollment, Cox proportional hazard regression analysis (47) was done to examine the relative risk (RR) of developing CIN3 by baseline detection of HPV16 or HPV18 variants. In this analysis, time to event was measured from the date of study entry to the onset of CIN3. The onset of event was defined as the midpoint between the visit at which the CIN3 was initially diagnosed and the most recent preceding visit. Women who did not develop CIN3 were censored at their last visit date. In addition to the time-fixed covariates described above for the analyses of risk of prevalence, we also included HPV16 or HPV18 status at the beginning of each of the 6-month intervals

as a time-dependent variable. To assess potential effects of the study arm-related missed or delayed diagnoses of prevalent CIN3 on estimates of risk by HPV16 variants, separate longitudinal analyses were done in the women who were enrolled in the immediate colposcopy and HPV triage arms and in the women who were enrolled in the conservative management arm.

A one-way ANOVA test was used to estimate differences in the mean length of follow-up by HPV16 and HPV18 variants. A χ^2 test, or Fisher's exact test when appropriate, was used to compare characteristics of the study subjects across HPV16 and HPV18 variants, and the 2-year prevalence of CIN3 by HPV16 and HPV18 variants and racial groups. All statistical tests were at the 5% two-sided significance level.

Results

HPV16 variants were evaluated in samples from 796 women, with HPV16 European, Asian, North American, Asian American, African-1, and African-2 variants detected in 654 (82.1%), 6 (0.8%), 6 (0.8%), 61 (7.7%), 36 (4.5%), and 33 (4.1%) samples, respectively. Because the number of infections with Asian or North American variants was so small (only six for each), these variants were excluded from the following analyses. As shown in Table 1, >50% of the women with HPV16 European or Asian American variants reported currently using hormonal contraceptives, compared with <40% of women with African-1 or African-2 variants ($P = 0.02$). Women with African-1 variants were less likely to be current smokers ($P = 0.04$). HPV18 Asian American, European, and African variants were detected in samples from 79 (28.5%), 119 (43.0%), and 79 (28.5%) women, respectively. More than 45% of the women with HPV18 Asian American and European variants reported current use of hormonal contraceptives; this proportion was 30% in women with African variants ($P < 0.01$). There were no appreciable differences in distribution of HPV16 or HPV18 variants by lifetime number of male sex partners, referral cytology, and number of routine Pap tests in the last 5 years.

Risk of Prevalent High-Grade CIN Associated with HPV16 and HPV18 Variants. Over a 2-year follow-up, CIN3 was histologically confirmed in 291 (37.4%) of the 779 women who had a baseline infection with HPV16 variants, including 201 first diagnosed at enrollment, 47 during follow-up, and 45 at exit. Compared with women with HPV16 European variants, the OR for the 2-year prevalence of CIN3 was 1.8 for those with HPV16 African-2 variants (95% CI, 0.9-3.8) or Asian American variants (95% CI, 1.0-3.1), after adjusting for study arm, self-reported race, and age, current use of hormonal contraceptives, and current smoking status at enrollment (Table 2). CIN2 was histologically confirmed in 107 women. The risk associations remained similar when \geq CIN2 was used as the clinical end point (data not shown).

Among 275 women with HPV18 infection, CIN3 was histologically confirmed in 47 (17.1%), including 29 initially diagnosed at enrollment, 3 during follow-up, and 15 at exit. Compared with women with African variants, the OR for the 2-year prevalence of CIN3 was 1.7 (95% CI, 0.6-4.7) for women with HPV18 European variants and 2.1 (95% CI, 0.7-6.3) for women with HPV18 Asian American variants, after adjusting for study arm, self-reported race, and age, current use of hormonal contraceptives, and current smoking status at enrollment (Table 2). CIN2 was histologically confirmed in 35 women. With \geq CIN2 as the end point, the adjusted ORs associated with HPV18 European and Asian American, compared with African, variants were 1.8 (95% CI, 0.8-4.2) and 2.6 (95% CI, 1.1-6.3), respectively.

The mean (\pm SD) lengths of follow-up were 21.3 (\pm 8.2), 20.6 (\pm 8.3), 24.3 (\pm 2.3), and 22.4 (\pm 6.9) months for women with HPV16 European, African-1, African-2, and Asian American variants, respectively ($P = 0.13$); and 21.9 (\pm 7.0), 23.3 (\pm 5.6), and 22.5 (\pm 7.5) months for women with HPV18 European, African, and Asian American variants, respectively ($P = 0.37$). To account for the effect of the length of follow-up on the risk of cervical neoplasia, we further examined the risk association using Cox regression analyses.

Risk for High-Grade CIN Subsequent to Baseline Detection of HPV16 and HPV18 Variants. To examine risk for developing CIN3 subsequent to baseline detection of

Table 1. Distribution of HPV16 and HPV18 variants by characteristics of the study subjects

Variables	No. (%) infection with HPV16*				P	No. (%) infection with HPV18*			P
	European variants (n = 654)	African-1 variants (n = 36)	African-2 variants (n = 33)	Asian American variants (n = 61)		European variants (n = 119)	African variants (n = 79)	Asian American variants (n = 79)	
Age at study entry									
18-19	104 (15.9)	5 (13.9)	6 (18.2)	18 (29.5)	0.19	17 (14.3)	9 (11.4)	14 (17.7)	0.09
20-24	305 (46.6)	20 (55.6)	18 (54.5)	28 (45.9)		67 (56.3)	34 (43.0)	38 (48.1)	
25-29	155 (23.7)	8 (22.2)	6 (18.2)	12 (19.7)		16 (13.4)	15 (19.0)	18 (22.8)	
\geq 30	90 (13.8)	3 (8.3)	3 (9.1)	3 (4.9)		19 (16.0)	21 (26.6)	9 (11.4)	
No. lifetime male sex partners†									
0-5	309 (48.0)	24 (66.7)	17 (51.5)	29 (47.5)	0.18	54 (45.8)	44 (56.4)	31 (40.8)	0.14
\geq 6	335 (52.0)	12 (33.3)	16 (48.5)	32 (52.5)		64 (54.2)	34 (43.6)	45 (59.2)	
Current use of hormonal contraceptives‡									
No	317 (49.0)	23 (63.9)	22 (66.7)	23 (37.7)	0.02	57 (47.9)	55 (69.6)	42 (54.5)	0.01
Yes	330 (51.0)	13 (36.1)	11 (33.3)	38 (62.3)		62 (52.1)	24 (30.4)	35 (45.5)	
Current smoking									
No	352 (53.8)	28 (77.8)	20 (60.6)	34 (55.7)	0.04	64 (53.8)	51 (64.6)	46 (58.2)	0.32
Yes	302 (46.2)	8 (22.2)	13 (39.4)	27 (44.3)		55 (46.2)	28 (35.4)	33 (41.8)	
Referral cytology									
ASCUS	402 (61.5)	21 (58.3)	15 (45.5)	35 (57.4)	0.30	62 (52.1)	38 (48.1)	39 (49.4)	0.87
LSIL	252 (38.5)	15 (41.7)	18 (54.5)	26 (42.6)		57 (47.9)	41 (51.9)	40 (50.6)	
No. Pap tests/y in the past 5 y§									
<1	399 (61.1)	17 (47.2)	15 (46.9)	37 (60.7)	0.17	67 (56.8)	37 (46.8)	37 (48.1)	0.31
\geq 1	254 (38.9)	19 (52.8)	17 (53.1)	24 (39.3)		51 (43.2)	42 (53.2)	40 (51.9)	

*A subject was double counted if she was positive for both HPV16 and HPV18 variants.

†Excluded were 15 women (10 with HPV16 infection and 5 with HPV18 infection) who did not provide information on number of lifetime sex partners.

‡Excluded were nine women (seven with HPV16 infection and two with HPV18 infection) who did not provide information on use of hormonal contraceptives.

§Excluded were five women (two with HPV16 infection and three with HPV18 infection) who did not provide information on Pap smear in the past 5 yrs.

Table 2. ORs and 95% CIs for prevalent CIN3 diagnoses associated with baseline infection with HPV16 and HPV18 variants

Types	Variants	No. subjects*	No. (%) with CIN3	Crude OR (95% CI)	Adjusted OR [†] (95% CI)
HPV16	European	649	237 (36.5)	1.0	1.0
	African-1	36	10 (27.8)	0.7 (0.3-1.4)	1.0 (0.4-2.2)
	African-2	33	15 (45.5)	1.4 (0.7-2.9)	1.8 (0.9-3.8)
HPV18	Asian American	61	29 (47.5)	1.6 (0.9-2.7)	1.8 (1.0-3.1)
	African	79	10 (12.7)	1.0	1.0
	European	118	20 (16.9)	1.4 (0.6-3.2)	1.7 (0.6-4.7)
	Asian American	78	17 (21.8)	1.9 (0.8-4.5)	2.1 (0.7-6.3)

*Excluded were seven women (five with HPV16 infection and two with HPV18 infection) who had a cytologic diagnosis of HSIL at baseline but no histologic evaluation and who did not return for follow-up. Additionally excluded were 12 women with either HPV16 Asian or North American variants. A subject was double counted if she was positive for both HPV16 and HPV18 variants.

[†]Adjusted for study arm, self-reported race, and age, current use of hormonal contraceptives, and current smoking status at enrollment.

HPV16 variants, we excluded 278 women who had CIN2-3 at enrollment and 51 women who did not provide any follow-up visits, leaving 455 in the analysis. CIN3 was histologically confirmed in 83 (18.2%) of these women. Compared with women with HPV16 European variants, the RR for developing CIN3 was 2.7 (95% CI, 1.0-7.0) for those with African-2 variants and 3.1 (95% CI, 1.6-6.0) for those with Asian American variants, after adjusting for study arm, self-reported race, and age, current use of hormonal contraceptives, and current smoking status at enrollment, as well as a time-dependent variable of HPV16 DNA status (Table 3). The increased risk of subsequent CIN3 associated with HPV16 African-2 or Asian American variants remained when restricting the analysis to the women who were enrolled in the immediate colposcopy and HPV triage arms (RR_{adjusted}, 4.3; 95% CI, 1.4-13.2) or to the women in the conservative management arm (RR_{adjusted}, 2.7; 95% CI, 1.4-5.4).

For the evaluation of risk for developing CIN3 subsequent to baseline detection of HPV18 variants, we excluded 47 women who had CIN2-3 at enrollment and 11 women who did not provide any follow-up visits, leaving 219 in the analysis. CIN3 was histologically confirmed in 18 (8.2%) of these women. Relative to women with HPV18 African variants, the risk of developing CIN3 was 3.8-fold greater for those with European variants (95% CI, 0.9-17.2) and 4.8-fold greater for those with Asian American variants (95% CI, 1.0-23.6; Table 3). Seventeen women developed CIN2 during follow-up. With \geq CIN2 as the end point, the adjusted RR associated with HPV18 European and Asian American, compared with African, variants was 2.8 (95% CI, 1.0-8.1) and 3.5 (95% CI, 1.1-10.6), respectively.

The 2-Year Prevalence of CIN3 by HPV16 and HPV18 Variants, Stratified by Racial Group. Of 774 HPV16-positive women with self-reported race information and histologic evaluation, 573 (74.0%) were White, 159 (20.5%) were African American, and 42 (5.4%) were American Indian/Alaskan or Asian/Pacific Islander. As shown in Table 4, the 2-year prevalence of CIN3 was higher in White women with

HPV16 European variants than in African American women with European variants ($P = 0.01$); a similar trend was observed among women with African-2 variants ($P = 0.07$). For those with African-1 variants, however, the trend was reversed, with a prevalence of 10% seen in White women and 35% in African American women ($P = 0.22$).

Of 274 HPV18-positive women with self-reported race information and histologic evaluation, 167 (60.9%) were White, 96 (35.0%) were African American, and 11 (4.0%) were American Indian/Alaskan or Asian/Pacific Islander. Relative to none of the 15 White women with HPV18 African variants having a diagnosis of prevalent CIN3, CIN3 was identified in 16% of the 62 African American women with African variants ($P = 0.19$; Table 4). Among those with HPV18 European variants, however, the prevalence of CIN3 seemed to be slightly higher in White women than in African American women (20.2% versus 8.7%, $P = 0.36$).

There was no appreciable difference in diagnoses of prevalent CIN3 between White and African American women who were infected with either HPV16 Asian American variants or HPV18 Asian American variants. However, among 42 American Indian/Alaskan or Asian/Pacific Islander women with HPV16 infections, CIN3 was identified in 8 (22%) of the 36 with European variants and in 3 (60%) of the 5 with Asian American variants. One Asian/Pacific Islander woman with a HPV16 African-2 variant had a diagnosis of ASCUS. Among 11 American Indian/Alaskan or Asian/Pacific Islander women with HPV18 infections, CIN3 was identified in one (25%) of the four with Asian American variants but none of those with African ($n = 2$) or European ($n = 5$) variants.

The trend of higher 2-year prevalence of CIN3 in women with HPV16 African-2 or Asian American compared with European variants, and in those with HPV18 European or Asian American compared with African variants was generally consistent across the strata of age, current smoking status, and current use of hormonal contraceptive at enrollment (data not shown).

Table 3. Subsequent risk of biopsy-confirmed CIN3 associated with baseline infection with HPV16 and HPV18 variants among women without CIN2-3 at study entry

Types	Variants	No. of women at baseline*	No. of person-months at risk	No. with CIN3 (per 1000 person-months)	Crude RR (95% CI)	Adjusted RR [†] (95% CI)
HPV16	European	374	7,886	62 (7.9)	1.0	1.0
	African-1	25	554	4 (7.2)	0.9 (0.3-2.5)	1.4 (0.5-4.0)
	African-2	22	468	5 (10.7)	1.3 (0.5-3.3)	2.7 (1.0-7.0)
	Asian American	34	635	12 (18.9)	2.5 (1.3-4.6)	3.1 (1.6-6.0)
HPV18	African	67	1,586	4 (2.5)	1.0	1.0
	European	95	2,145	9 (4.2)	1.7 (0.5-5.5)	3.8 (0.9-17.2)
	Asian American	57	1,322	5 (3.8)	1.5 (0.4-5.7)	4.8 (1.0-23.6)

*A subject was double counted if she was positive for both HPV16 and HPV18 variants.

[†]Adjusted for study arm, self-reported race, and age, current use of hormonal contraceptives, and current smoking status at enrollment, in addition to a time-dependent variable of detection of HPV16 DNA over time for analysis of risk by HPV16 variants and of HPV18 DNA over time for analysis of risk by HPV18 variants.

Table 4. Prevalent CIN3 diagnoses by HPV16 and HPV18 variants stratified by racial group

Types	Variants	White women		African American women		<i>P</i> *
		No. subjects	No. (%) with CIN3	No. subjects	No. (%) with CIN3	
HPV16	European	500	197 (39.4)	108	28 (25.9)	0.01
	African-1	10	1 (10.0)	26	9 (34.6)	0.22
	African-2	15	10 (66.7)	17	5 (29.4)	0.07
	Asian American	48	23 (47.9)	8	3 (37.5)	0.71
HPV18	African	15	0 (0.0)	62	10 (16.1)	0.19
	European	90	18 (20.2)	23	2 (8.7)	0.36
	Asian American	62	13 (21.0)	11	2 (18.2)	1.00

*Probability of seeing a difference as extreme as that observed due solely to chance in prevalent CIN3 diagnoses between White and African American women who were infected with HPV16 European, African-1, African-2, or Asian American variants; and between White and African American women who were infected with HPV18 African, European or Asian American variants.

Discussion

In this study of ALTS participants who had baseline infections with HPV16 or HPV18, we found that an increased risk of high-grade CIN was associated with HPV16 African-2 and Asian American compared with European variants and with HPV18 European and Asian American compared with African variants. The associations were not explained by factors previously shown to be related to risk for cervical neoplasia, including age, race, smoking, and use of hormonal contraceptives, or by different managements of the study arms. Because characterization of the variants was done without knowledge of any clinical or epidemiologic information and cervical histology was diagnosed by the panel of expert pathologists before detection of the variants, the potential for ascertainment bias was minimized. It is also unlikely that the risk associations resulted from differential access to health care, because ALTS participants came from a screened population of women who were referred to the trial due to an abnormal Pap smear; in this well-controlled trial setting, all of them were examined, followed, and diagnosed according to the study protocol.

Considering the insensitivity of identifying prevalent cases by only a baseline measurement, we used CIN3 histologically diagnosed at any study visit as the clinical end point in the initial analyses. This approach minimizes potential biases introduced by delayed or missed diagnoses at baseline. One concern, however, is that not all women had the same length of follow-up. If the length of follow-up had been differentially related to the variants and clinical outcomes, the risk estimates could have been biased. Thus, the cohort analysis of risk for CIN3 subsequent to the baseline infection was also done in women without \geq CIN2 at entry. Further, we are aware that in the cohort analysis, a portion of follow-up diagnoses of high-grade CIN was actually missed prevalent cases. If these cases were differentially related to the variants, biases could have occurred. Although we were unable to distinguish CIN3 cases missed at baseline from those that truly developed during follow-up, conducting separate analyses among women in the immediate colposcopy and HPV triage arms and among women in the conservative management arm allowed us to compare the subsequent risks between those who underwent colposcopy and biopsy at enrollment and those who did so only if cytologic evidence of HSIL was detected. The consistency between the prevalent and subsequent risks associated with HPV16 and HPV18 variants supports the hypothesis that the variants differ in their oncogenic potentials.

The reduced oncogenic potential associated with HPV16 European variants has been reported previously from several case-control (27, 28) and longitudinal studies (24-26). In these studies, however, all non-European lineages were usually lumped due to a small number of infections. The present study not only provides powerful confirmation of the risk associa-

tion because of its large sample size but also extends previous findings by showing that, specifically, the African-2 and Asian American variants contributed to the increased risk of CIN3. Data on the clinical relevance of HPV18 variants are very limited. Although an unbalanced distribution of HPV18 variants between women with and without cervical cancer has been reported previously, the difference was not statistically assessed (37). To our knowledge, the present report is one of the first, if not the first, study to examine the independent effects of HPV18 variants on risk of high-grade CIN in a longitudinal setting. As shown in the results, the HPV18 Asian American lineage, also termed prototype, represented high-risk variants whereas the African lineage represented low-risk variants. It should be noted that historical classification of HPV16 European and HPV18 Asian American lineages as the prototype for these respective types was based on the preceding of viral isolation rather than knowledge of their disease risk or biological properties.

Importantly, our data further suggest race-associated differences in risk for prevalent CIN3 by certain variants. For those with HPV16 European variants, White women were more likely to have CIN3 than were African American women. For those with HPV16 African-1 variants, however, African American women tended to have a higher proportion of prevalent CIN3 diagnoses than did White women. Similar risk patterns associated with the European versus African variants by racial groups were also observed among women with HPV18 infections. Although the differences were not statistically significant (except for the risk by HPV16 European variants), the reverse risk trend in the prevalence of CIN3 associated with these variants between White and African American women underscores the role of race in defining the variant-related risk and warrants further investigation.

The underlying mechanism for the race-associated risk difference is presently unclear but it may be related to the effectiveness of the host's immune response. In our previous study of race-associated viral DNA persistence (38), we found that HPV16 and HPV18 European variants persist longer in White women and African variants persist longer in African American women. The prolonged persistence of HPV16 African variants seen in African American women was attributable to the African-1 variants. The European variants may have an advantage over African variants in evading the host immune surveillance in White women (and vice versa in African American women). Because persistent, compared with transient, infection with oncogenic HPV types increases the risk of cervical diseases (39-42), it is likely that the race-associated difference in the risk of CIN3 between infections with HPV16 European and African-1 variants and between infections with HPV18 European and African variants may in part be explained by abilities of these variants to establish persistent infection by circumventing the host's immune responses.

Although the host-variant-related immune response seems to be a plausible explanation for the risk differences between HPV16 European and African-1 variants and between HPV18 European and African variants, this theory alone would not, however, sufficiently account for the increased risk of CIN3 associated with the African-2 and Asian American variants. Although our previous analyses of viral persistence (38) indicated that the European variants were somewhat more likely than African-2 variants to persist in White women, the 2-year prevalence of CIN3 tended to be higher in White women with HPV16 African-2 variants than in White women with European variants. In addition, among White women, although the Asian American variants did not differ substantially from other variants in terms of viral persistence (data not shown), the increased risk of high-grade CIN seemed to be related to infection with HPV16 and HPV18 Asian American variants compared with HPV16 European and HPV18 African variants, respectively. Clearly, other attributes of the variants may also play a role in defining the risk of CIN3.

Indeed, the variable oncogenic potentials of HPV16 and HPV18 variants are suggested by *in vitro* studies showing that the variants differ in their abilities to induce p53 degradation, keratinocyte differentiation, and the E2-related transcription (48, 49) and that the Asian American, compared with the European, variants carry strong promoter activities that drive transcription of E6/E7 oncogenes (50, 51). In agreement with this, HPV18 Asian American, compared with African, variants presented an increased ability of inducing tumor formation *in vivo* (50). It is possible that some nucleotide alterations may directly alter the oncogenic potentials of the variants and may be responsible for the observed risk differences. We are aware that, although tempting, speculation of function-related nucleotide alterations is beyond the scope of the present study because the sequence variation identified in this study also reflects cosegregation of alterations in other regions. Nevertheless, the findings from this study help to tag a group of variants that confer an increased risk of CIN3.

Last, we noted a marginally significant difference in the 2-year prevalence of CIN3 between White and African American women who were infected with HPV16 African-2 variants, although a likelihood of persistence of the African-2 variants was similar between these women (38). The reason for this is presently unclear. We hypothesize that race-associated cellular proteins other than those related to immune presentation may interact differently with the African-2 variants in HPV-involved pathogenesis. We are still far from understanding the behavior of the variants *in vivo*, which is much more complicated than the situation *in vitro* because of viral-host and viral gene-gene interactions.

Several limitations of the study should be addressed. Although this study included the largest number of HPV16 and HPV18 infections to date, the numbers of study subjects (and clinical events) in some racial strata were quite small, particularly for White women with African variants and African American women with Asian American variants. Thus, the findings of race-specific risks by HPV16 and HPV18 variants should be interpreted with great caution. Also, the present study included a large number of CIN3 cases initially detected at enrollment. Although there is no *a priori* reason to believe that the presence of disease could itself make particular variants be more detectable, bias could be introduced if certain variants were associated with persistence of cervical lesions. Although this cannot be ruled out, the consistency between the prevalent and subsequent risks associated with HPV16 and HPV18 variants strongly argues against this possibility. Finally, the 2-year follow-up period may not have been sufficient to fully examine the late effects of these infections. Presently, however, there are no data to suggest differences between the short- and long-term risks by HPV16 or HPV18 variants.

In summary, our data showed that the increased risk of high-grade CIN was associated with HPV16 African-2 and Asian American variants compared with European variants and with HPV18 European and Asian American variants compared with African variants. Host-variant-related viral persistence and/or changes to the biological properties of the virus due to certain nucleotide alterations may explain the risk association.

Acknowledgments

We thank the ALTS Group for providing the biological specimens and clinical trial data, and Marsha Weese for help in manuscript preparation.

References

1. Stewart AC, Eriksson AM, Manos MM, et al. Intratype variation in 12 human papillomavirus types: a worldwide perspective. *J Virol* 1996;70:3127–36.
2. Calleja-Macias IE, Kalantari M, Huh J, et al. Genomic diversity of human papillomavirus-16, 18, 31, and 35 isolates in a Mexican population and relationship to European, African, and Native American variants. *Virology* 2004;319:315–23.
3. Calleja-Macias IE, Villa LL, Prado JC, et al. Worldwide genomic diversity of the high-risk human papillomavirus types 31, 35, 52, and 58, four close relatives of human papillomavirus type 16. *J Virol* 2005;79:13630–40.
4. Prado JC, Calleja-Macias IE, Bernard HU, et al. Worldwide genomic diversity of the human papillomaviruses-53, 56, and 66, a group of high-risk HPVs unrelated to HPV-16 and HPV-18. *Virology* 2005;340:95–104.
5. Chan SY, Ho L, Ong CK, et al. Molecular variants of human papillomavirus type 16 from four continents suggest ancient pandemic spread of the virus and its coevolution with humankind. *J Virol* 1992;66:2057–66.
6. Ong CK, Chan SY, Campo MS, et al. Evolution of human papillomavirus type 18: an ancient phylogenetic root in Africa and intratype diversity reflect coevolution with human ethnic groups. *J Virol* 1993;67:6424–31.
7. Yamada T, Manos MM, Peto J, et al. Human papillomavirus type 16 sequence variation in cervical cancers: a worldwide perspective. *J Virol* 1997;71:2463–72.
8. Arias-Pulido H, Peyton CL, Torrez-Martinez N, Anderson DN, Wheeler CM. Human papillomavirus type 18 variant lineages in United States populations characterized by sequence analysis of LCR-E6, E2, and L1 regions. *Virology* 2005;338:22–34.
9. Giannoudis A, Herrington CS. Human papillomavirus variants and squamous neoplasia of the cervix. *J Pathol* 2001;193:295–302.
10. Munoz N, Bosch FX, de Sanjose S, et al.; International Agency for Research on Cancer Multicenter Cervical Cancer Study Group. Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N Engl J Med* 2003;348:518–27.
11. Clifford GM, Gailus S, Herrero R, et al. IARC HPV Prevalence Surveys Study Group. Worldwide distribution of human papillomavirus types in cytologically normal women in the International Agency for Research on Cancer HPV prevalence surveys: a pooled analysis. *Lancet* 2005;366:991–8.
12. Londesborough P, Ho L, Terry G, Cuzick J, Wheeler C, Singer A. Human papillomavirus genotype as a predictor of persistence and development of high-grade lesions in women with minor cervical abnormalities. *Int J Cancer* 1996;69:364–8.
13. Andersson S, Alemi M, Rylander E, et al. Uneven distribution of HPV 16 E6 prototype and variant (L83V) oncoprotein in cervical neoplastic lesions. *Br J Cancer* 2000;83:307–10.
14. Zehbe I, Voglino G, Delius H, Wilander E, Tommasino M. Risk of cervical cancer and geographical variations of human papillomavirus 16 E6 polymorphisms. *Lancet* 1998;352:1441–2.
15. Zehbe I, Wilander E, Delius H, Tommasino M. Human papillomavirus 16 E6 variants are more prevalent in invasive cervical carcinoma than the prototype. *Cancer Res* 1998;58:829–33.
16. Kammer C, Tommasino M, Syrjanen S, et al. Variants of the long control region and the E6 oncogene in European human papillomavirus type 16 isolates: implications for cervical disease. *Br J Cancer* 2002;86:269–73.
17. Etherington IJ, Ellis JR, Luesley DM, Moffitt DD, Young LS. Histologic and immunologic associations of an HPV16 variant in LoSIL smears. *Gynecol Oncol* 1999;72:56–9.
18. Nindl I, Rindfleisch K, Lotz B, Schneider A, Durst M. Uniform distribution of HPV 16 E6 and E7 variants in patients with normal histology, cervical intra-epithelial neoplasia and cervical cancer. *Int J Cancer* 1999;82:203–7.
19. van Duin M, Snijders PJ, Vossen MT, et al. Analysis of human papillomavirus type 16 E6 variants in relation to p53 codon 72 polymorphism genotypes in cervical carcinogenesis. *J Gen Virol* 2000;81:317–25.
20. Kurvinen K, Yliskoski M, Saarikoski S, Syrjanen K, Syrjanen S. Variants of the long control region of human papillomavirus type 16. *Eur J Cancer* 2000;36:1402–10.
21. Hu X, Guo Z, Tianyun P, et al. HPV typing and HPV16 E6-sequence

- variations in synchronous lesions of cervical squamous-cell carcinoma from Swedish patients. *Int J Cancer* 1999;83:34–7.
22. Luxton J, Mant C, Greenwood B, et al. HPV16 E6 oncogene variants in women with cervical intraepithelial neoplasia. *J Med Virol* 2000;60:337–41.
 23. Bontkes HJ, van Duin M, de Gruijl TD, et al. HPV 16 infection and progression of cervical intra-epithelial neoplasia: analysis of HLA polymorphism and HPV 16 E6 sequence variants. *Int J Cancer* 1998;78:166–71.
 24. Xi LF, Koutsky LA, Galloway DA, et al. Genomic variation of human papillomavirus type 16 and risk for high grade cervical intraepithelial neoplasia. *J Natl Cancer Inst* 1997;89:796–802.
 25. Villa LL, Sichero L, Rahal P, et al. Molecular variants of human papillomavirus types 16 and 18 preferentially associated with cervical neoplasia. *J Gen Virol* 2000;81:2959–68.
 26. Xi LF, Carter JJ, Galloway DA, et al. Acquisition and natural history of human papillomavirus type 16 variant infection among a cohort of female university students. *Cancer Epidemiol Biomarkers Prev* 2002;11:343–51.
 27. Hildesheim A, Schiffman MH, Bromley C, et al. Human papillomavirus type 16 variants and risk of cervical cancer. *J Natl Cancer Inst* 2001;93:315–8.
 28. Berumen J, Ordóñez RM, Lazcano E, et al. Asian-American variants of human papillomavirus 16 and risk for cervical cancer: a case-control study. *J Natl Cancer Inst* 2001;93:1325–30.
 29. Schlecht NF, Burk RD, Palefsky JM, et al. Variants of human papillomaviruses 16 and 18 and their natural history in human immunodeficiency virus-positive women. *J Gen Virol* 2005;86:2709–20.
 30. Rajeevan MS, Swan DC, Nisenbaum R, et al. Epidemiologic and viral factors associated with cervical neoplasia in HPV-16-positive women. *Int J Cancer* 2005;115:114–20.
 31. Beskow AH, Engelmark MT, Magnusson JJ, Gyllensten UB. Interaction of host and viral risk factors for development of cervical carcinoma *in situ*. *Int J Cancer* 2005;117:690–2.
 32. Hording U, Teglbaerg CS, Visfeldt J, Bock JE. Human papillomavirus types 16 and 18 in adenocarcinoma of the uterine cervix. *Gynecol Oncol* 1992;46:313–6.
 33. Teshima H, Beaudenon S, Koi S, et al. Human papillomavirus type 18 DNA sequences in adenocarcinoma and adenosquamous carcinoma of the uterine cervix. *Arch Gynecol Obstet* 1997;259:169–77.
 34. Burk RD, Terai M, Gravitt PE, et al. Distribution of human papillomavirus types 16 and 18 variants in squamous cell carcinomas and adenocarcinomas of the cervix. *Cancer Res* 2003;63:7215–20.
 35. Lizano M, Berumen J, Guido MC, Casas L, Garcia-Carranca A. Association between human papillomavirus type 18 variants and histopathology of cervical cancer. *J Natl Cancer Inst* 1997;89:1227–31.
 36. De Boer MA, Peters LA, Aziz MF, et al. Human papillomavirus type 18 variants: histopathology and E6/E7 polymorphisms in three countries. *Int J Cancer* 2005;114:422–5.
 37. Hecht JL, Kadish AS, Jiang G, Burk RD. Genetic characterization of the human papillomavirus (HPV) 18 E2 gene in clinical specimens suggests the presence of a subtype with decreased oncogenic potential. *Int J Cancer* 1995;60:369–76.
 38. Xi LF, Kiviat NB, Hildesheim A, et al. Human papillomavirus type 16 and 18 variants: racial-related distribution and persistence. *J Natl Cancer Inst* 2006;98:1045–52.
 39. Ho GY, Burk RD, Klein S, et al. Persistent genital human papillomavirus infection as a risk factor for persistent cervical dysplasia. *J Natl Cancer Inst* 1995;87:1365–71.
 40. Schlecht NF, Kulaga S, Robitaille J, et al. Persistent human papillomavirus infection as a predictor of cervical intraepithelial neoplasia. *JAMA* 2001;286:3106–14.
 41. Ferenczy A, Franco E. Persistent human papillomavirus infection and cervical neoplasia. *Lancet Oncol* 2002;3:11–6.
 42. Kjaer SK, van den Brule AJ, Paull G, et al. Type specific persistence of high risk human papillomavirus (HPV) as indicator of high grade cervical squamous intraepithelial lesions in young women: population based prospective follow up study. *BMJ* 2002;325:572.
 43. Carbillet JP, Kantelip B, Schaal JP, Mougin C. Persistence and load of high-risk HPV are predictors for development of high-grade cervical lesions: a longitudinal French cohort study. *Int J Cancer* 2003;106:396–403.
 44. Schiffman MH, Adriaan ME. ASCUS-LSIL Triage Study. Design, methods and characteristics of trial participants. *Acta Cytol* 2000;44:726–42.
 45. ASCUS-LSIL Triage Study (ALTS) Group. Results of a randomized trial on the management of cytology interpretations of atypical squamous cells of undetermined significance. *Am J Obstet Gynecol* 2003;188:1383–92.
 46. Lemeshow S, Hosmer DW, Jr. Estimating odds ratios with categorically scaled covariates in multiple logistic regression analysis. *Am J Epidemiol* 1984;119:147–51.
 47. Cox DR. Regression models and life tables. *J R Stat Soc (Series B)* 1972;34:187–220.
 48. Stoppler MC, Ching K, Stoppler H, Clancy K, Schlegel R, Icenogle J. Natural variants of the human papillomavirus type 16 E6 protein differ in their abilities to alter keratinocyte differentiation and to induce p53 degradation. *J Virol* 1996;70:6987–93.
 49. Ordóñez RM, Espinosa AM, Sanchez-Gonzalez DJ, Armendariz-Borunda J, Berumen J. Enhanced oncogenicity of Asian-American human papillomavirus 16 is associated with impaired E2 repression of E6/E7 oncogene transcription. *J Gen Virol* 2004;85:1433–44.
 50. De la Cruz-Hernandez E, Garcia-Carranca A, Mohar-Betancourt A, et al. Differential splicing of E6 within human papillomavirus type 18 variants and functional consequences. *J Gen Virol* 2005;86:2459–68.
 51. Sichero L, Franco EL, Villa LL. Different P105 promoter activities among natural variants of human papillomavirus type 18. *J Infect Dis* 2005;191:739–42.