Asian-American Variants of Human Papillomavirus 16 and Risk for Cervical Cancer: a Case–Control Study

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Background: **Human papillomavirus 16 (HPV16) has a number of variants, each with a different geographic distribution and some that are associated more often with invasive neoplasias. We investigated whether the high incidence of cervical cancer in Mexico (50 cases per 100 000 women) may be associated with a high prevalence of oncogenic HPV16 variants.** ***Methods:*** **Cervical samples were collected from 181 case patients with cervical cancer and from 181 age-matched control subjects, all from Mexico City. HPV16 was detected with an E6/E7 gene-specific polymerase chain reaction, and variant HPV classes and subclasses were identified by sequencing regions of the E6 and L1/MY genes. Clinical data and data on tumor characteristics were also collected. All statistical tests were two-sided.** ***Results:*** **HPV16 was detected in cervical scrapes from 50.8% (92 of 181) of case patients and from 11% (20 of 181) of control subjects. All HPV16-positive samples, except one, contained European (E) or Asian-American (AA) variants. AA and E variants were found statistically significantly more often in case patients (AA = 23.2% [42 of 181]; E = 27.1% [49 of 181]) than in control subjects (AA = 1.1% [two of 181]; E = 10% [18 of 181]) (*P*<.001 for case versus control subjects for either E or AA variants, χ^2 test). However, the frequency of AA variants was 21 times higher in cancer patients than in control subjects, whereas that ratio for E variants was only 2.7 (*P* = .006, χ^2 test). The odds ratio (OR) for cervical cancer associated with AA variants (OR = 27.0; 95% confidence interval [CI] = 6.4 to 113.7) was higher than that associated with E variants (OR = 3.4; 95% CI = 1.9 to 6.0). AA-positive case patients (46.2 ± 12.5 years [mean ± standard deviation]) were 7.7 years younger than E-positive case patients (53.9 ± 12.2 years) (*P* = .004, Student's *t* test). AA variants were associated with squamous cell carcinomas and adenocarcinomas, but E variants were associated with only squamous cell carcinomas (*P* = .014, Fisher's exact test).** ***Conclusions:*** **The high frequency of HPV16 AA variants, which appear to be more oncogenic than E variants, might contribute to the high incidence of cervical cancer in Mexico. [J Natl Cancer Inst 2001;93: 1325–30]**

Cervical cancer is one of the most common cancers in women. Incidence rates of this disease vary from about 10 cases per 100 000 women per year in many industrialized nations to more than 40 per 100 000 in some developing countries *(1).* Of the half a million cases of cervical cancer estimated annually in the world, nearly 80% occur in developing countries *(2).* Mexico has one of the highest incidence rates of cervical cancer (50 cases per 100 000 women), and 16 000 new cases are detected every year *(3,4).* The high incidence of this disease may reflect a poor screening program *(5)* and differences in the human papillomavirus (HPV) infecting the Mexican population. Various types of HPV are associated with 90%–100% of cervical cancers worldwide *(2,6),* and HPV16 is detected in about 50%. To our knowledge, essentially no variation in HPV positivity and viral types among countries with high and low incidences of cervical cancer has been described *(1,7).* Some HPV variants, which differ from the reference viral-type sequence by up to 2.0%, have been associated with high-grade cervical intraepithelial neoplastic lesions *(8,9),* invasive cervical carcinomas *(10,11),* or more aggressive cervical cancers *(12).* The incidence of cervical cancer in different countries may be associated with the distribution of specific viral variants, since HPV variants are distributed differently among geographic regions. For instance, HPV16 variants are distributed differently among the five continents *(13):* Asian-American (AA) variants are located mainly in Central and South America and Spain, African variants are mainly in Africa, Asian variants are mainly in Southeast Asia, and European (E) variants are in all regions other than Africa.

Genetic variation among HPV16 variants has been found in the E6, E7, L1, L2, E5, and E2 genes and in the long control region. The sequence variation or mutation after infection may modify the function of the encoded protein, as shown for L1 mutations that affect viral assembly *(14)* and some E6 variants that differ in their ability to immortalize cells and to degrade p53 *(15).* We have shown previously *(16)* that HPV16 AA variants are detected frequently in Mexican patients with cervical cancer, that the E2 gene in these variants contains extensive nucleotide changes, and that infected cells have a high viral copy number and retain the E1/E2 genes.

In this report, by using a case–control study of women in Mexico City, we investigate the frequency of HPV16 variants (classes and subclasses) in patients with cervical cancer and in control subjects and the association of variants with clinical data and with the risk of cervical cancer.

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Subjects and Methods

Samples

A total of 181 patients with invasive cervical cancer diagnosed at the Gynecologic Obstetric Hospital "Castelazo Ayala" of the Mexican Institute of Social Security in Mexico City were recruited. Case patients were obtained sequentially during the period from June 1997 through May 1999 and represent about 20% of the patients with newly diagnosed cervical cancer in this period because of the restrictive inclusion criteria of our study (to have resided in Mexico City at least 1 year, to have received no previous treatment, to be incident cases, and to come from family medicine units of the geopolitical area of study). Among the 181 cervical cancers, 84% were squamous cell carcinomas, 6.6% were adenocarcinomas, 5% were adenosquamous carcinomas, and 4.4% were undifferentiated carcinomas.

A total of 181 age-matched control subjects (within 2 years of the case patient's age; range = 26–88 years) were selected from women attending the Cervical Cancer Screening Program, Preventive Medicine Service, Mexican Institute of Social Security, from the same area and during the same period as the case patients. When one case patient was identified and confirmed, an age-matched control subject with a normal Pap smear was chosen from the screening program of the same medical clinic. The participation rate of case and control subjects was close to 95%, which is consistent with previous studies in Mexico *(17,18).*

Informed written consent was obtained from all participants, and the study protocol was approved by the local ethics committee. The case subjects received a complete clinical evaluation. Tumor staging was done according to the last international revised protocol for gynecologic cancer *(19).* Colposcopically directed biopsy specimens were obtained from the case patients. Tissues were fixed in formalin, embedded in paraffin, and stained with hematoxylin–eosin for routine pathologic diagnosis. For HPV detection and typing, in case patients and control subjects, a cervical scrape from the endocervix and ectocervix was collected with a cytobrush, and the cells were suspended in a vial with extraction buffer *(20)* and stored at –20 °C until analysis. One hundred case patients in this study had data available from both cervical scrapes and tumor biopsy examination. For HPV16 detection, agreement was 98% between these types of specimens, and no difference was found for variants detection.

Detection of HPV16 and Characterization of HPV16 Variants

DNA was purified by phenol extraction. HPV16 was detected blindly in case and control specimens by polymerase chain reaction (PCR) with specific primers for E6/E7 region, as described previously *(21).* The L1 region of HPV16-positive samples was amplified by a universal PCR with the use of MY09/MY11 primers as described previously *(22).* HPV16 classes and subclasses were identified by sequencing the entire MY region and E6 gene in both directions with the fluorescent cycle-sequencing method (BigDye Terminator ready reaction kit; Perkin-Elmer, Branchburg, NJ) as described previously *(16).* Sequence analysis was performed with an ABI PRISM 310 genetic analyzer system (Perkin-Elmer). Data were analyzed with DNASIS software (Hitachi Software Engineering Co., Ltd., Yokohama, Japan). HPV16 sequences and base positions were numbered according to the 1997 sequence database (Los Alamos National Laboratory, Los Alamos, NM), and variant designation was done according to Yamada et al. *(13).*

Statistical Analysis

For patient age, the results were expressed as the mean ± standard deviation (SD) of the number (n) of observations indicated. Student's *t* test was used to assess the statistical significance of differences in age among groups. A χ^2 test or Fisher's exact test was used, as appropriate, to assess the statistical significance of differences in the frequency of HPV16 variants among age, histologic, or clinical groups. The effects of clinical stage, histologic type, and HPV16 variants on the mean age of the subjects were assessed by analysis of variance (ANOVA). Differences were considered to be statistically significant when *P* values were less than .05. To evaluate the association between HPV16 classes and/or subclasses and cervical cancer, we used a conditional logistic regression model, and we estimated specific odds ratios (ORs) with 95% confidence intervals (CIs). The attributable fraction (AF) was calculated as follows: AF = prevalence of the variant in the population of cancer × (1 – 1/OR). The database was managed with FoxPro software (Microsoft Corporation, Redmond, WA), and STATA software programs (Stata Corporation, College Station, TX) were used for statistical analyses. All statistical tests were two-sided.

Results

Frequency of AA Variants in Cervical Carcinomas From Women in Mexico City

Among the 181 invasive cervical carcinomas studied, 92 (50.8%) were positive for HPV16. The most prevalent variants were the E variant class E-350G (23.8%, 43 of 181) and the AA class (23.2%, 42 of 181). Four subclasses of the E-350G class, two subclasses of the E-350T class, and two subclasses of the AA class (AA-a and AA-c) were identified (Table 1). DNA sequences from all AA-c variant isolates were identical to the DNA sequence of isolate IS.53, and DNA sequences from all AA-a isolates were identical to the DNA sequence of isolate OR.8160 *(13).* Among all 92 HPV16 isolates, 86 (93.5%) had a guanine at position 350 and six (6.5%) had a thymine at position 350. HPV16 was detected in 20 (11.0%) of 181 control subjects, 18 from E class (17 were E-350G and one was E-350T) and two from AA class (one was subclass AA-c and one was subclass AA-a) (Table 1). The frequency of AA variants was 21 times higher in the cancer group (23.2% [42 of 181]) than in the control group (1.1% [two of 181]), whereas the frequency of E variants was only 2.7 times higher in the cancer group (27.1% [49 of 181]) than in the control group (10% [18 of 181]) ($P = .006$ for AA versus E, χ^2 test; Table 2).

Table 1. Frequency of human papillomavirus 16 (HPV16) class/subclasses in samples from case patients with cervical cancer and control subjects in Mexico*

Class/subclass	Frequency (%)	
	Control subjects (n = 181)	Cancer patients (n = 181)
HPV16		
Positive	20 (11.0)	92 (50.8)
Negative	161 (89.0)	89 (49.2)
Class		
AA	2 (1.1)	42 (23.2)
E-350G	17 (9.4)	43 (23.8)
E-350T	1 (0.6)	6 (3.3)
NA1	0	1 (0.6)
Subclass		
AA-a	1 (0.6)	22 (12.2)
AA-c	1 (0.6)	20 (11.0)
E-P350G	14 (7.7)	33 (18.2)
E-C188G	3 (1.7)	6 (3.3)
E-G131G	0	1 (0.6)
E-A176G	0	3 (1.7)
E-P350T	1 (0.6)	5 (2.8)
E-A176T	0	1 (0.6)

*AA = Asian-American; E = European; NA = North American. HPV16 class/subclass designation is according to Yamada et al. *(13).* Values for percents were rounded to one decimal place. Note that the NA1 subclass was not included.

Age and Prevalence of AA and E Variants of HPV16

AA-class-positive case patients (46.2 ± 12.5 years [mean ± standard deviation]; range = 28–72 years) were 7.7 years younger than E-class-positive case patients (53.9 ± 12.2 years; range = 29–86 years) ($P = .004$, Student's *t* test). The mean ages of control subjects positive for AA variants (34.5 ± 12.0 years) and for E variants (47.2 ± 13.4 years) were not statistically significantly different ($P = .22$, Student's *t* test). Case patients positive for E subclass variants had the same mean age. When we compared the ages of AA-positive case patients grouped by variant subclass, the age difference between the patients with HPV16 AA and E classes increased primarily because of the age of AA-c-positive case patients (42.4 ± 9.5 years; range = 29–60 years; $P<.001$, Student's *t* test), whereas the age difference between the AA-a-positive case patients (49.6 ± 14 years; range = 28–72 years) and the E-positive case patients de-

Table 2. Frequency and ORs of E and AA variants of human papillomavirus 16 (HPV16) in cancer and control groups*

					HPV16 classes					
		HPV16			E			AA		
Group	No.	% (n)	OR	95% CI	% (n)	OR	95% CI	% (n)	OR	95% CI
Control	181	11.0 (20)	1.0†	—	10.0 (18)	1.0†	—	1.1 (2)	1.0†	—
Cancer	181	50.8 (92)	8.3	4.8 to 14.4	27.1 (49)	3.4	1.9 to 6.0	23.2 (42)	27.0	6.4 to 113.7

*E = European; AA = Asian-American; OR = odds ratio; CI = confidence interval. $P = .006$ for E versus AA in all groups, two-sided χ^2 test.

†Referent.

creased. No statistically significant difference was observed in the mean age of patients in the two AA subclasses. When case patients were grouped into two categories by age (≤35 years old and >35 years old), HPV16 variants were found to be differentially distributed (Table 3). In the younger group, AA variants (58.8%, 10 of 17) were more frequently detected than E variants (11.8%, two of 17) ($P = .012$, χ^2 test). In the older group, the frequency of E variants (28.7%, 47 of 164) and of AA variants (19.5%, 32 of 164) was not statistically significantly different ($P = .07$, χ^2 test). The distribution of AA variants was statistically significantly different between both age groups ($P<.001$, Fisher's exact test). Furthermore, the distribution of the AA-a and AA-c variants was not uniform in the older group. Among those 36–60 years old (n = 118), 15 were AA-c positive; however, among those older than 60 years (n = 46), none was AA-c positive ($P = .006$, Fisher's exact test). AA-a-positive patients were similarly distributed in these older age groups. Differences in the mean ages among case patients positive for different variant groups were statistically significant, even after adjustment for differences in histologic groups ($P = .008$, ANOVA) or clinical stages ($P = .025$, ANOVA).

Table 3. Distribution of human papillomavirus 16 (HPV16) variants by age in patients with cervical cancer (n = 181)*

	Frequency (%)	
HPV16	≤35 y old (n = 17)	>35 y old (n = 164)
AA	10 (58.8)	32 (19.5)
E	2 (11.8)	47 (28.7)
NA	0	1 (0.6)
Negative	5 (29.4)	84 (51.2)

*AA = Asian-American; E = European; NA = North American. $P = .012$ for AA versus E in the younger group, two-sided χ^2 test. $P<.001$ for distribution of AA in both age groups, two-sided Fisher's exact test.

Association of Variants With Histologic Types of Cervical Cancer

HPV16 in general was detected at similar frequencies (50%) among the histologic types of cervical carcinoma. However, HPV16 variants were not. AA and E variants were detected at similar frequencies in squamous cell carcinomas (23.0% and 29.6%, respectively), but only AA variants were detected in adenocarcinomas (50%) ($P = .014$ for AA versus E in adenocarcinomas, Fisher's exact test; Table 4). AA and E variants were detected with similar frequencies in all clinical stages.

HPV16 Variants and Risk of Cervical Cancer

The increased frequency of AA variants in the cancer group compared with the control group and the associations of AA variants with young patients and adenocarcinomas suggest that AA variants are more oncogenic than E variants. It is interesting that AA variants have an OR of 27.0 for cervical cancer (95% CI = 6.4 to 113.7), which is 3.3 and 7.9 times higher than that of HPV16 in general (OR = 8.3; 95% CI = 4.8 to 14.4) and HPV16 E classes (OR = 3.4; 95% CI = 1.9 to 6.0), respectively (Table 2). When analyzed separately, the ORs of AA-a and AA-c subclasses were similar. Thus, HPV16 AA classes are associated with a much higher risk of cervical cancer than are HPV16 E classes. The AF of cervical cancers due to AA variants was 22.3% and due to E variants was 19.2%.

The ORs of HPV16 variants were compared in case patients 35 years old or younger and in those older than 35 years. In both groups, AA variants had similar ORs (OR = 34.3 [95% CI = 3.7 to 316.1] and OR = 37.6 [95% CI = 5.1 to 278.7], respectively), but AA variants had much higher ORs than E variants (OR = 0.98 [95% CI = 0.15 to 6.6] in the younger group and OR = 3.8 [95% CI = 2.0 to 7.1] in the older group). The AF of cervical cancer due to AA class was 57.1% in the younger group and 19.0% in the older group. No cervical cancer in the younger group was attributable to E variants, and only 21.1% in the older group was attributable to E variants.

Discussion

We have found that HPV16 AA variants conferred a higher risk for cervical cancer than HPV16 E variants and that almost a quarter of all cervical cancers in Mexico can be attributed to AA variants. The high incidence of cervical cancer in Mexico may be explained by the poor coverage of the Pap screening program, which reaches only 30% of adult women. However, the high frequency of HPV16 AA variants, which appear more oncogenic than E variants, might also contribute to the high incidence of cervical cancer in Mexican women.

The makeup of the control group might be a limiting factor in this study because HPV16 has a very low OR for cervical cancer (OR = 8.3) and because the control group has a high prevalence of HPV16 (11.0%). This high prevalence may reflect a problem in the control group design, a problem in the cytologic diagnosis from control cervical tissue samples

Table 4. Distribution of human papillomavirus 16 (HPV16) and HPV16 variants between squamous cell carcinomas and adenocarcinomas

	Frequency: No. positive/No. tested (%)		
Cell type	HPV16	HPV16 AA*	HPV16 E*
Squamous cell carcinoma	80/152 (52.6)	35/152 (23.0)	45/152 (29.6)
Adenocarcinoma	6/12 (50)	6/12 (50)	0/12 (0)

*AA = Asian-American; E = European. $P = .014$ for AA versus E in adenocarcinomas, two-sided Fisher's exact test.

(scrapes), or the actual prevalence of the virus in Mexican women who do not have cervical lesions. For logistic and practical reasons, we chose a clinic-based control group, which included women with a normal Pap smear, who were matched (one to one) with case patients by age and time of selection and who were from the screening program at the same medical clinic. Therefore, both samples could be assumed to have come from the same cohort and to be homogeneous as far as sociodemographic features and HPV infection exposure were concerned. Two additional linked factors may affect the prevalence of HPV16 in the control group. First, half of the women with cervical neoplasia went to screening because they had gynecologic symptoms *(17).* Second, Mexico has a high proportion of false-negative results from Pap tests that can be as high as 50% under the best of conditions *(23).* However, according to the prevalence of Pap screening abnormalities found in this and previous studies [96.4% with normal Pap test, 2.1% with low-grade lesions, 1.2% with high-grade lesions, and 0.25% with invasive carcinoma *(18)*], the contribution of these two factors may be minor: If we assume that half of the abnormalities were reported as false negatives (3.55%) and that half of the false-negative specimens were HPV16 positive, these factors would contribute only 1.8% of HPV16-positive control subjects. On the other hand, because the prevalence of HPV16 found in this study is similar to that reported in population-based control subjects (13.2%) in Mexico City *(24),* our results may reflect the actual prevalence of HPV16 in women without cervical lesions in Mexico City. Population-based control subjects could be used to avoid most of the potential problems with the control group and the OR underestimation. However, the important findings of this study are the differences between AA and E variants in cervical carcinomas, which are not affected by the design of the control subject group.

The prevalence of HPV16 (50.8%) in our case patients is similar to that reported in other countries *(6,7).* The percentage of Mexican patients with cervical cancers testing positive for AA variants (45.7% of HPV16-positive samples) is much higher than that reported for Central and South America (20%), Europe (14%, all from Spain), and Asia (5.7%) *(13).* The differences are also statistically significant ($P<.001$ for Mexico versus Central and South America, Europe, or Asia, χ^2 test). Such differences come mainly from the AA-c variant, because this variant is found more frequently in Mexico (21.7%) than in Central and South America (4.4%), Europe (2%), and Southeast Asia (0%) ($P<.005$ for Mexico versus Central and South America, Europe, or Southeast Asia, χ^2 test) *(13).* The high prevalence of the AA-c variant in Mexico might result from differences in ethnic or genetic background. Compared with some Central and South American populations, the Mexican population has a larger proportion of individuals with American Indian ancestry and a smaller proportion with Spanish ancestry *(25).* Although the ethnicity of the Central and South American people studied by Yamada et al. *(13)* was not specified, in most of the countries studied (including Argentina, Brazil, Chile, Colombia, Cuba, Panama, and Paraguay), the European genetic component is greater than the Amerindian component. In fact, Ho et al. *(26)* suggested that variant types of HPV16 correlate with the ethnicity of populations rather than with geography. Because the AA-c variant is very common in Mexico and rare or absent elsewhere in the world *(13),* the AA-c variant may be associated with the Amerindian genetic background and could have arisen in the New World *(16).* AA-a and E variants could have been introduced and spread in Mexico by the Spanish conquistadors. The similar frequency of AA-a and AA-c in this study may result from the great admixture of Indians and Spaniards that began almost 500 years ago with the Spanish colonization of Mexico.

The frequency of the E variant E-350G in cervical cancer samples from Mexico (46.7% of HPV16-positive samples) is very similar to that found in Europe, Central and South America, and North America *(13),* but the frequency of the variant E-350T is statistically significantly much lower ($P<.001$, χ^2 test) in Mexico (6.5%) than in Europe (40%), Central and South America (24.6%), and North America (53%). It should be noted that the frequency of E-350G and E-350T variants in Mexico is the inverse of that in Southeast Asia *(13).* Thus, the increased frequency of AA variants may also reflect a decreased frequency of the E-350T variant, suggesting unique physiologic or immunologic adaptations of these variants in the mestizo Mexican population. It is also important to note that 93.5% of the cervical carcinomas (including those positive for AA, E-350G, and NA1 variants) analyzed had an E6 gene with a thymine-to-guanine base change at position 350, which has been associated with a particularly high risk for cervical cancer in some areas *(10)* but not in all *(11).*

Cervical cancer in women 35 years old or younger is apparently more aggressive than in older women, with earlier lymph node metastasis and decreased rates of survival *(27).* One explanation, the association of HPV18 with aggressive tumors, has been controversial *(28,29).* The incidence of cervical cancer in young women has been increasing during the last two decades in many countries except in the United States *(30)* and, in one report *(31),* this increased incidence is associated with race. Thus, as suggested for Mexico, differences might be the result of Pap screening programs or the prevalence rates of specific HPV16 variants *(13).* As our data indicated, AA variants could explain almost 60% of the cervical carcinomas in Mexican women 35 years old or younger.

The association of AA variants with younger women, adenocarcinomas, and a higher risk for cervical cancer, as observed in our study, strongly suggests that AA variants are more oncogenic than E variants. The higher oncogenicity observed with AA variants could result from the increased neoplastic activity of E6/E7 oncogenes, more efficient viral replication, or better stimulation of early viral gene expression. The following clinical and experimental data support this hypothesis: Xi et al. *(8)* observed that women with HPV16 AA or other non-E variants had a 4.5 times greater risk of developing high-grade cervical intraepithelial neoplastic lesions than women with E variants. The E6 protein from an AA-a variant (isolate 512) consistently produces more serum and calcium differentiation-resistant colonies in primary human foreskin keratinocytes and stimulates p53 degradation better than the E variant E6 reference protein *(15).* The copy number of AA variants per cell is higher than that of E variants *(16),* suggesting that AA variants replicate better than E variants. The activity of the p97 promoter in AA-c variants was 3.3-fold higher than that of the E reference virus, suggesting increased expression of viral oncogenes *(32).* Since AA-c-positive patients are 11 years younger than E-positive patients,

the stronger activity or higher expression of AA-c E6/E7 oncogenes may lead to the early development of preinvasive lesions and then invasive cancer.

At similar stages, adenocarcinomas of the uterine cervix are usually more biologically aggressive and have a poorer prognosis than squamous cell carcinomas *(33,34).* Generally, HPV18 has been associated with adenocarcinomas and HPV16 has been associated with squamous cell carcinomas *(6,35).* In our cervical cancer samples, HPV18 was detected, as described previously *(21),* in 15.1% (23 of 152) of squamous cell carcinomas and in 33.3% (four of 12) of adenocarcinomas (data not shown). More than twice as many HPV18-positive adenocarcinomas were detected than HPV18-positive squamous cell tumors, but the difference was not statistically significant. The high frequency of HPV16-positive adenocarcinomas could explain the lack of an association between HPV18 and adenocarcinomas in our study. It is interesting that, in our study, when all HPV16-positive adenocarcinomas were typed, only AA variants were detected; thus, even with the small sample size, the association of AA variants with adenocarcinomas was strong ($P = .014$, Fisher's exact test). Furthermore, we believe that the reported low frequency of HPV16 in adenocarcinomas can be explained geographically. Because HPV16 AA variants are rarely detected in any region of the world except for Mexico, Central and South America, and Spain, the association of adenocarcinomas with HPV18 could be observed clearly, but that with HPV16 would not be detected. At present, there is no clear explanation for the association between tumor histology and HPV type. However, in a previous report *(12),* we did find an association between particular HPV18 variants and specific histologic types of cervical cancers (var-2 with squamous cell carcinomas and HPV18 reference with small-cell carcinomas and adenocarcinomas).

If amino acid changes within non-E variants are located in epitopes critical for the immune response, vaccines developed against E variants may have a reduced efficacy in countries where those non-E variants are present at high frequency. Therefore, in addition to HPV types, the prevalence of HPV variants should be considered when designing the appropriate HPV vaccine for a specific area.

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NOTES

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