

Age-Related Changes of the Cervix Influence Human Papillomavirus Type Distribution

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Abstract

Approximately 15 human papillomavirus (HPV) types cause virtually all cervical cancer whereas other HPV types are unrelated to cancer. We were interested in whether some noncarcinogenic types differ from carcinogenic in their affinity for the cervical transformation zone, where nearly all HPV-induced cancers occur. To examine this possibility, we tested cervical specimens from 8,374 women without cervical precancer and cancer participating in a population-based study in Guanacaste for >40 HPV types using PCR. We compared age-group specific prevalences of HPV types of the $\alpha 9$ species, which are mainly carcinogenic and include HPV16, to the genetically distinct types of the $\alpha 3/\alpha 15$ species (e.g., HPV71), which are noncarcinogenic and common in vaginal specimens from hysterectomized women. We related HPV detection of each group to the location of the junction between the squamous epithelium of the ectocervix and vagina and the columnar epithelium of the endocervical canal. Models evaluated the independent effects of amount of exposed columnar epithelium (ectopy) and age on the presence of $\alpha 9$ or $\alpha 3/\alpha 15$ types. Prevalence of $\alpha 9$ types (7.6%) peaked in the youngest women, declined in middle-aged women, and then increased slightly in older women. By contrast, prevalence of $\alpha 3/\alpha 15$ types (7.6%) tended to remain invariant or to increase with increasing age. Detection of $\alpha 9$ infections increased ($P_{\text{trend}} < 0.0005$) but $\alpha 3/\alpha 15$ infections decreased ($P_{\text{trend}} < 0.0005$) with increasing exposure of the columnar epithelia. Older age and decreasing cervical ectopy were independently positively associated with having an $\alpha 3/\alpha 15$ infection compared with having an $\alpha 9$ infection. These patterns need to be confirmed in other studies and populations. We suggest that these genetically distinct groups of HPV types may differ in tissue preferences, which may contribute to their differences in carcinogenic potential. (Cancer Res 2006; 66(2): 1218-24)

Introduction

Over 100 human papillomaviruses (HPV) types infect humans and these types are primarily classified in the α and β genera (1, 2).

Note: Consent was obtained from all participants in accordance with the guidelines of U.S. Department of Health and Human Services. NIH and Costa Rica institutional review boards approved this study.

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Approximately 40 HPV types sorting into 15 α species infect mucosal epithelia, and approximately 15 carcinogenic HPV types from 5 α species cause virtually all cervical cancers (3–5) and a subset of head and neck, vaginal, vulvar, penile, and anal cancers worldwide (6, 7). Differences in carcinogenicity have been primarily ascribed to sequence-related functional differences in E6 and E7 proteins and their interaction with a variety of cellular proteins but most notably with p53 and Rb, respectively (8, 9). In addition, carcinogenic HPV types code for an E5 protein, implicated in carcinogenic transformation (9) and immune evasion (10), whereas many noncarcinogenic types either lack an open reading frame (ORF) or the necessary start codon for E5 expression (11). Thus, several mechanisms may simultaneously contribute to the carcinogenic potential of HPV.

Another plausible mechanism to explain differences in carcinogenicity is viral tropism for the target cell type. Profound examples of papillomavirus tropism exist: animal papillomaviruses do not infect human tissues and HPV types primarily of the β species infect skin (some types of the α species also infect skin) rather than mucosal epithelia. However, it is unclear whether subtle differences in tropism between α species populated by carcinogenic HPV types (e.g., $\alpha 9$) versus noncarcinogenic HPV types (e.g., $\alpha 3$ and $\alpha 15$) occur. Most cervical cancers arise in the cervical transformation zone, a region of squamous metaplasia between the proximal squamous epithelia found in the vagina and ectocervix and the columnar epithelia in the endocervix. Thus, for the cervical cancer to possibly occur, infection by carcinogenic types must occur in the tissue of the cervical transformation zone.

We recently reported a comparative analysis of vaginal HPV in hysterectomized women and cervical HPV in nonhysterectomized women from our population-based study in Guanacaste, Costa Rica (12). We found that (a) carcinogenic HPV types were similarly prevalent in both groups of women; (b) some noncarcinogenic HPV types, especially those types within $\alpha 3$ species (e.g., HPV61 and HPV72) and the closely related $\alpha 15$ species (HPV71), were more common in the vaginal specimens from hysterectomized women than cervical specimens in nonhysterectomized women; and (c) the difference in noncarcinogenic HPV type prevalences was primarily observed in women 55 years of age and younger. Data from cervicovaginal specimens collected by self-sampling have found an increase in prevalence of these same $\alpha 3/\alpha 15$ types compared with cervical specimens (13, 14). Together, we hypothesized that some HPV types might preferentially infect the squamous epithelium of the vagina rather than the metaplastic cells of the cervical transformation zone, where HPV-induced cancer primarily occurs.

If our hypothesis is true, we could anticipate that the physiologic age of the cervix (i.e., aging-related atrophy of the epithelial layer) and “migration” of the cervical transformation zone and the

squamocolumnar junction, the distal boundary of the cervical transformation zone, into the cervical canal due to aging (15) may influence prevalence of HPV types when the cervix is sampled at the os. That is, changes in the location of the cervical transformation zone, using the change of the squamocolumnar junction as an anatomical marker, may influence the distribution of cell types collected at the os of the cervix from where cells are collected for cytology and HPV DNA testing. In this circumstance, cell samples from the cervical os of a younger woman, characterized by a more exposed cervical transformation zone (greater cervical ectopy) and visible squamocolumnar junction, may have a greater fraction of squamous metaplastic and columnar cells than the cells collected from older women, which will have a greater fraction of squamous cells (lesser or no cervical ectopy). Such an age-related sampling bias could influence our epidemiologic studies of HPV infection and HPV DNA testing.

In this analysis, we examined whether there was epidemiologic evidence that cervical ectopy influenced patterns of HPV prevalence of nonhysterectomized women in our Guanacaste study, thereby providing stronger evidence of HPV-type viral tropism for squamous or metaplastic epithelia. Based on the evidence in our analysis of hysterectomized women (12), we focused our analysis on the patterns of HPV prevalence for types of the $\alpha 3/\alpha 15$ species compared with those of the $\alpha 9$ species, which are populated by common and predominantly carcinogenic HPV types including HPV16, the most carcinogenic HPV type (3–5, 16).

Materials and Methods

Study population. Detailed methods of this National Cancer Institute (NCI)- and local Institutional Review Board-approved population-based study in Guanacaste, Costa Rica have been reported elsewhere (17, 18). Briefly, after selecting a random sample of censal segments of this mainly rural population (240,000 inhabitants), we did a house-to-house enumeration of resident adult women (≥ 18 years), identifying a target population of 11,742 potentially eligible subjects. From June 1993 to December 1994, we invited potential participants to visit local clinics for an appointment with our staff and recruited 10,049 women who agreed to participate and to sign an informed consent.

Data and specimen collection. Specially trained study nurses did a pelvic exam on sexually active women (9,175 women examined, 97% of the eligible), which included cervical cell collections for cytology and HPV DNA testing (17, 18). We considered 8,374 women in this analysis after excluding women diagnosed with cervical intraepithelial neoplasia of ≥ 2 at baseline to rule out disease effects on HPV prevalence ($n = 139$), women without a cervix because of hysterectomy ($n = 630$), and women without PCR results ($n = 32$).

Detection and genotyping of HPV DNA. We used a MY09/M11 L1 consensus primer PCR (MY09/11 PCR) method for amplification of HPV DNA as previously described (19, 20). PCR products were typed using dot blot hybridization for 48 types as previously described (19, 20). We categorized HPV61, HPV71, HPV72, HPV81, HPV83, HPV84, and HPV89 as $\alpha 3/\alpha 15$ species types and HPV16, HPV31, HPV33, HPV35, HPV52, HPV58, and HPV67 as $\alpha 9$ species types (1, 2). In preliminary analyses, we separately evaluated $\alpha 7$ types (HPV18, HPV39, HPV45, HPV59, HPV68, and HPV70). Types of $\alpha 4$ and $\alpha 14$ species, which are closely related to $\alpha 3$, were not included because either we did not test for those types (HPV2, HPV27, and HPV90) or we did not test for them individually (HPV57).

Image analysis of the cervix. During the Guanacaste Project, two cervigrams (National Testing Laboratories Worldwide, Fenton, MO) were taken during all patient visits. Cervigrams (35-mm slides) were scanned using Super CoolScan 4000 ED scanner (Nikon, Inc., Melville, NY) at 1,660 dots per inch resolution and stored in Tagged Image File Format. Digitized pictures were then converted to JPEG format using a compression ratio of 40:1.

The digitized pictures were evaluated on a 17-in. color monitor using the Boundary Marking Tool software developed by the Division of Cancer Epidemiology and Genetics and the National Library of Medicine. This software enabled the evaluator to draw boundaries around the anatomic areas of the cervix and convert them into pixels. The evaluator (J.J.) selected the better of two cervigram images for the evaluation.

We selected a subset of all 945 women with an $\alpha 9$ infection ($n = 534$) and/or an $\alpha 3/\alpha 15$ infection ($n = 513$; 102 women were coinfecting with types from both HPV groups) and for whom there was a cervigram image to examine the relationships of cervical ectopy with the detection of these starkly contrasting HPV species groups. The locations of the squamocolumnar junction and the cervical os were drawn using a Graphire2 mouse (Wacom Technology Corp., Vancouver, WA). The annulus of area bounded by the drawn squamocolumnar junction and cervical os was considered as a qualitative measure of the area (degree) of cervical ectopy (i.e., the relative amount of glandular tissue exposed to cell sampling). This area was then converted to pixels by an algorithm written by an engineer from National Library of Medicine (L.R.L.). On a set of 77 cervigrams reevaluated in masked fashion within a larger set of cervigrams evaluated, we found that our continuous measurement of ectopy was highly reproducible (Spearman correlation = 0.99).

Statistical analysis. To set the stage for the assessment of the transformation zone, we calculated the age group-specific (<25, 25-34, 35-44, 45-54, 55-64, 65-74, and ≥ 75 years) HPV prevalences for each individual type of the $\alpha 3/\alpha 15$, $\alpha 9$, and $\alpha 7$ species. We then calculated the prevalences of $\alpha 3/\alpha 15$ and $\alpha 9$ in aggregate as a group with binomial 95% confidence intervals (95% CI). Pearson χ^2 was used to test for statistical differences ($P < 0.05$) in the overall group prevalence by age group.

We next related the degree of cervical ectopy to detection of $\alpha 9$ or $\alpha 3/\alpha 15$ infection, restricted to women who had infections with types from either or both groups. By eliminating women from consideration that had neither group, we tried to concentrate on the contrast of interest. The degree of cervical ectopy as measured above was categorized as quartiles of area or as no squamocolumnar junction visible/no ectopy. These categories were then related to detection of either group of infections using Pearson χ^2 and Mantel extension test for trend. When we also stratified on age (<35, 35-49, and ≥ 50 years) to control for the effects of aging, we used broader categories of ectopy by combining finer categories based on similarities (no squamocolumnar junction, 1st and 2nd quartiles of area, and 3rd and 4th quartiles of area), which was necessary given the small numbers.

Finally, we contrasted the determinants of $\alpha 3/\alpha 15$ detection to those for $\alpha 9$ detection by using the latter group as the referent group. Standard contingency table methods were used to assess possible crude associations of data collected from a risk factor questionnaire that assessed information on sociodemographic characteristics; sexual, reproductive, and birth control practices; cigarette smoking; and sexually transmitted diseases with $\alpha 3/\alpha 15$ detection (versus $\alpha 9$ detection). Variables found to be univariately associated with $\alpha 3/\alpha 15$ detection were included in logistic regression models to calculate odds ratios (OR) and 95% CIs as measures of association. Dose-response relationships (P_{trend}) were assessed in the models by assuming a linear trend and treating ordinal variables as continuous.

Results

Of the 8,374 women included in this analysis, 633 women (7.6%; 95% CI, 7.0-8.1%) were positive for at least one $\alpha 9$ type and 77 of 633 women (12.1%; 95% CI, 9.0-15.0%) were positive for more than one $\alpha 9$ type. By comparison, 634 women (7.6%; 95% CI, 7.0-8.1%) were positive for at least one $\alpha 3/\alpha 15$ type and 64 of 634 women (10.1%; 95% CI, 7.9-12.7%) were positive for more than one $\alpha 3/\alpha 15$ type. Age-prevalence curves for the individual phylogenetically related types, $\alpha 9$ types (Fig. 1A) and $\alpha 3/\alpha 15$ types (Fig. 1B), showed distinctly different patterns. HPV types in $\alpha 9$ group had peak prevalences in the youngest women, as expected for a sexually transmitted infection, and secondary minor peaks in women of

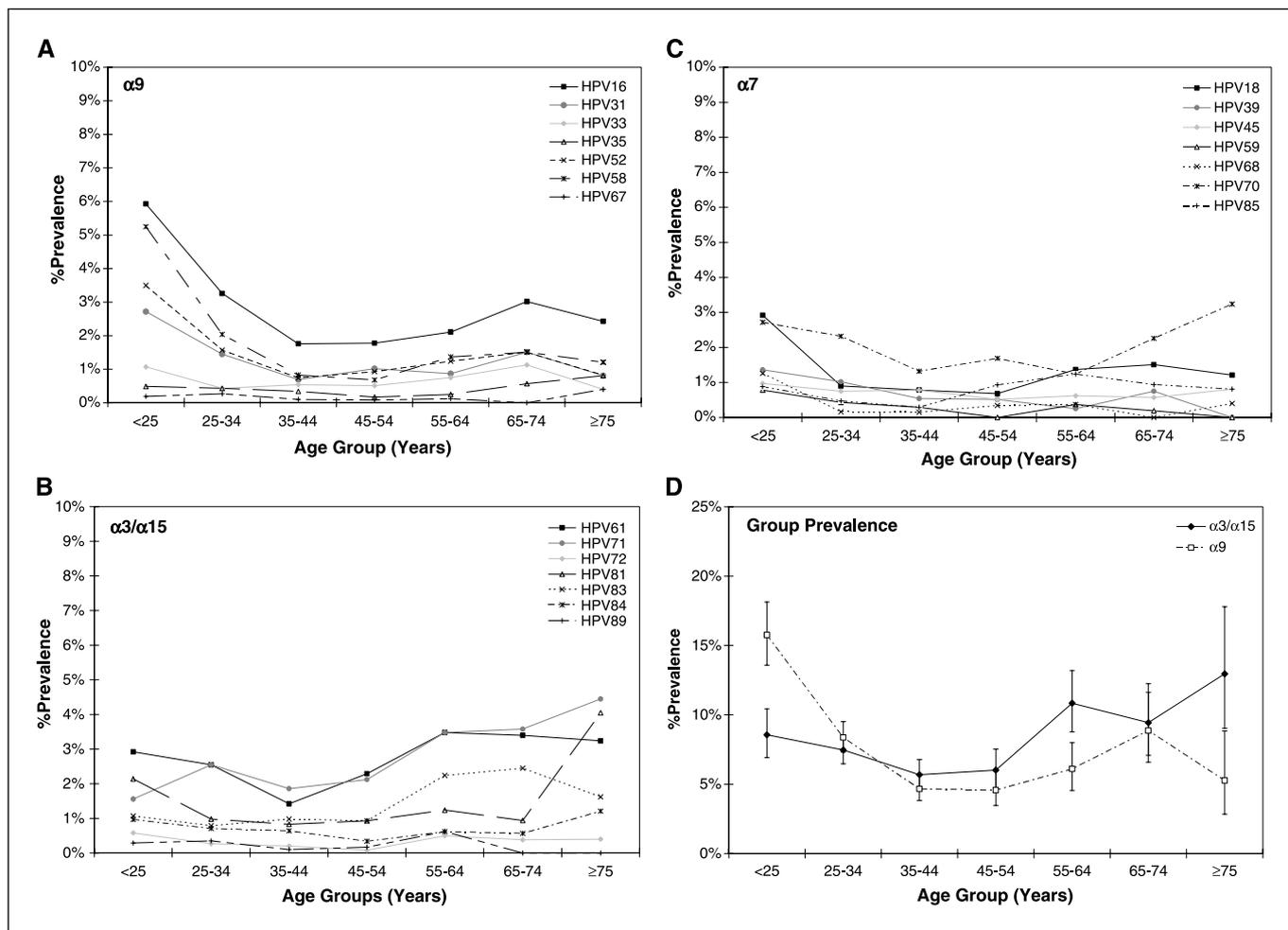


Figure 1. Age-group specific prevalences for individual HPV types of the α9 species (A), α3/α15 species (B), and α7 species (C) and any type of the α9 species (□) and α3/α15 species (◆; Bars, D binomial exact 95% confidence intervals). The scales for individual types and grouped types differ.

ages 55 to 64 years. By contrast, prevalence of α3/α15 types had a tendency to be either relatively constant or to increase with increasing age. The age patterns of types in the α7 species (Fig. 1C), a group of primarily carcinogenic types and which are more closely related genetically to α3/α15 than to α9, had peak prevalences in the youngest women and secondary minor peak in older women, especially HPV18. A notable exception for the α7 types was HPV70, which had a prevalence of 2.7% in women of ages <25 years and 3.2% in women of ages >75 years. Nevertheless, the most significant contrast was between α3/α15 and α9 types, and therefore we limited our subsequent analyses to these groups of types.

The combined prevalence (Fig. 1D) of the types in each group confirms the differences observed for the age-group prevalences of individual types. There was a significantly greater prevalence of α9 types in women of ages <25 years compared with the prevalence of α3/α15 types. Conversely, there was a significantly greater prevalence of α3/α15 types in women of ages 55 to 64 and ≥75 years compared with the prevalence of α9 types in women of the same age groups.

In a group of 945 women with α3/α15 and/or α9 type infections and available cervigram images, we evaluated relationships of α3/α15 and α9 positivity with the degree of cervical ectopy (Fig. 2). In this subset, there was a strong positive trend ($P_{\text{trend}} < 0.0005$)

of increasing α9 positivity with greater degrees of cervical ectopy, with the lowest α9 for the no squamocolumnar junction category (41.1%) and greatest α9 for the 3rd quartile of area (68.2%) and 4th (highest) quartile of area (61.7%; Fig. 2B). By contrast, there was a strong positive trend ($P_{\text{trend}} < 0.0005$) of α3/α15 positivity with lesser degrees of ectopy, with the lowest for the 4th (highest) quartile of area (46.8%) and 3rd quartile of area (44.5%) and the highest positivity peak for the no squamocolumnar junction category (68.7%).

Although age and degree of ectopy are highly correlated ($P_{\text{trend}} < 0.0005$), stratifying by broad categories of age did not change these contrasting patterns (Fig. 3). We found a trend of increasing detection of α9 types [$P_{\text{trend}} < 0.0005$; P (heterogeneity of linear trends between age groups) = 0.7] and decreasing detection of α3/α15 types [$P_{\text{trend}} = 0.004$; P (heterogeneity of linear trends) = 0.9] with increasing cervical ectopy.

Only age, oral contraceptive use (never, former, and current), and degree of ectopy were crudely associated with detection of α3/α15 versus α9; other variables, such as recent and lifetime numbers of partners, were not related to differences in detection of α3/α15 versus α9. Oral contraceptive use, as previously reported (15), was strongly associated with increased cervical ectopy ($P_{\text{trend}} < 0.0005$) even after stratifying on age [$P_{\text{trend}} < 0.0005$; P (heterogeneity of linear trends) = 0.2; Table 1]. However, its effect on HPV types was

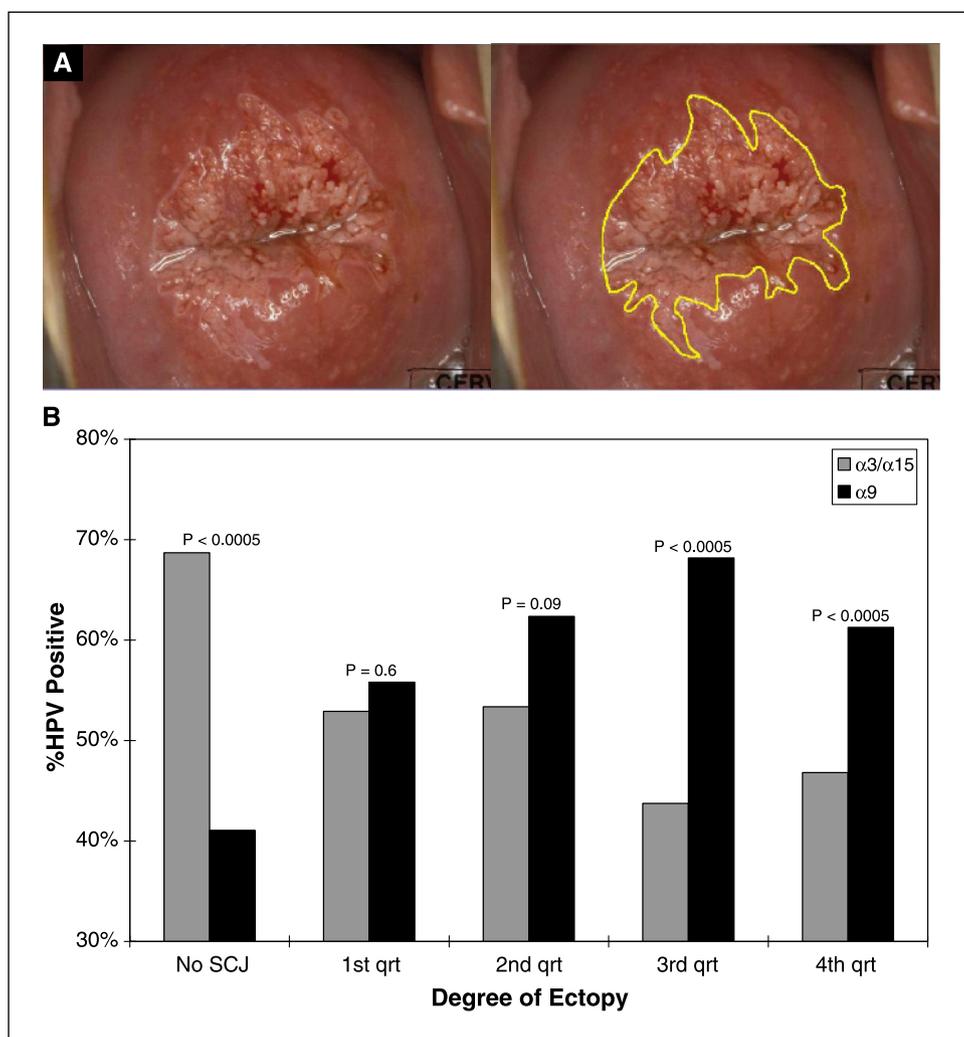


Figure 2. A, cervigram images of the cervix without and with a marked squamocolumnar junction using a boundary marking tool. B, comparison of the % positivity of $\alpha 3/\alpha 15$ (■) and $\alpha 9$ types (■) with degree of ectopy, as determined by the area demarcated by the marked squamocolumnar junction. *qrt*, quartile of ectopic area.

confounded by age and possibly mediated through ectopy. In a multivariable model (Table 2), age was positively associated ($P_{\text{trend}} = 0.002$) and ectopy was negatively associated ($P_{\text{trend}} = 0.009$) with $\alpha 3/\alpha 15$ positivity compared with $\alpha 9$ positivity. Oral contraceptive use was not associated with $\alpha 3/\alpha 15$ positivity.

Discussion

We observed a strong tendency of $\alpha 3/\alpha 15$ HPV types to be found in older cervical tissues composed predominantly of squamous epithelial cells compared with $\alpha 9$ HPV types, which are more commonly found in cancer-susceptible tissues of the cervical transformation zone that are composed of squamous metaplastic epithelial cells. Thus, the dynamic age-related changes in cervix influence HPV type-specific detection at the cervical os, where cervical specimens are routinely collected. One implication of these results is that the observed age-prevalence pattern for $\alpha 3/\alpha 15$ types in this and other studies might not reflect the true age pattern but is confounded by the age-dependent differences in composition of cells sampled at the cervical os as indicated by the associations with degree of ectopy. A second implication is that the increased presence of certain $\alpha 3/\alpha 15$ types (e.g., HPV61, HPV71, and HPV81) in older women may increase the likelihood of a false-positive test result by Hybrid Capture 2 (Digene Corp., Gaithers-

burg, MD), a U.S. Food and Drug Administration–approved test for carcinogenic HPV DNA, because of its imperfect type specificity for carcinogenic types (21).

We suggest two possible mechanisms for the observed patterns. First, vaginal squamous epithelia may be more susceptible than squamous metaplasia of the cervical transformation zone to infections by $\alpha 3/\alpha 15$ types. Therefore, $\alpha 3/\alpha 15$ types may tend to infect the squamous epithelia of the vagina in younger women and “migrate” into the os of the cervix as the cervical transformation zone, the front/distal edge of which is the squamocolumnar junction, recedes into the canal with aging. The fraction of squamous epithelial or “vaginal” cells would be expected to be small in young women and/or women with cervical ectopy compared with the fraction in older women and/or those with no ectopy.

The observed increase in prevalence in the $\alpha 3/\alpha 15$ types with aging supports this notion (see Fig. 3). Moreover, whereas prevalent infections by both groups of types are strongly associated with lifetime numbers of sexual partners, unlike $\alpha 9$ types, $\alpha 3/\alpha 15$ types are not associated with having multiple sexual partners in the last 6 months (data not shown). This is consistent with a delayed detection of $\alpha 3/\alpha 15$ types at the cervical os. However, the smaller increase in prevalence of the $\alpha 9$ types in older women weakens this explanation. Second, the cervical transformation zone may be more

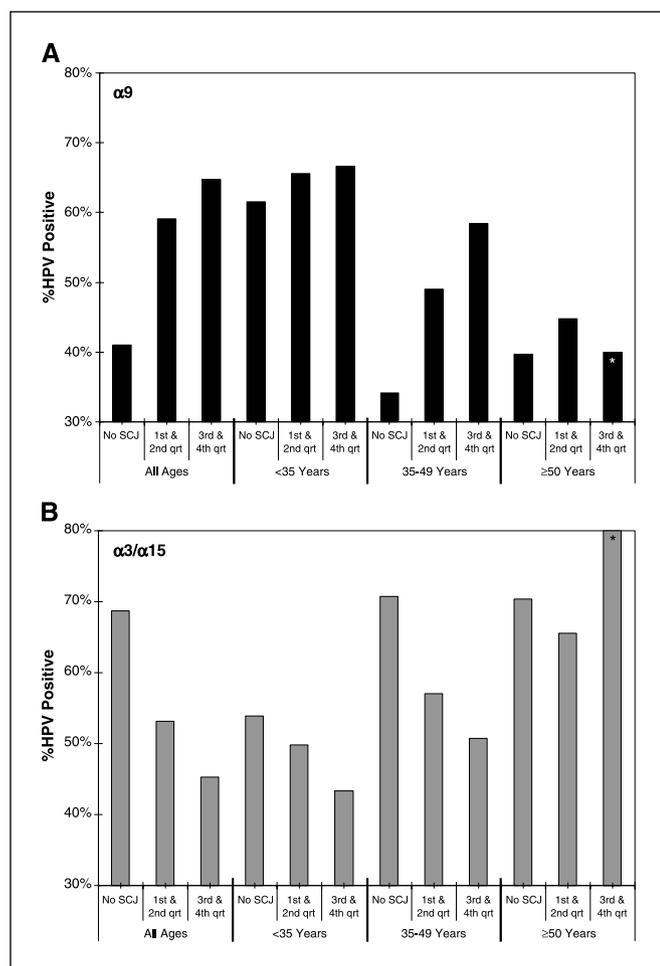


Figure 3. Age stratification of crude relationship of cervical ectopy and positivity for $\alpha 9$ (A) and $\alpha 3/\alpha 15$ types (B). *, only five women were in this category of age and degree of ectopy.

susceptible than vaginal squamous epithelia for infections by $\alpha 9$ types. The decrease in $\alpha 9$ positivity with decreasing cervical ectopy would suggest a preference for the cervical transformation zone over the vaginal squamous epithelia.

However, the similar prevalence of oncogenic HPV types in vaginal specimens from hysterectomized women and in cervical specimens from nonhysterectomized women (12) indicates a significant susceptibility of the vaginal epithelia to oncogenic HPV infection. The evidence is strongest for the first mechanism, predilection of $\alpha 3/\alpha 15$ types for vaginal epithelia. The difference in patterns of $\alpha 9$ in women with increasing cervical ectopy is not easily reconciled with the similar prevalences observed in vaginal specimens from hysterectomized and cervical specimens from nonhysterectomized women, although biases between these two groups cannot be excluded. Moreover, there is evidence to suggest that vulvovaginal infection by non- $\alpha 3/\alpha 15$ HPV types precedes, and therefore may serve as reservoir for, cervical infection (22).

It is uncertain whether the observed distinctions between $\alpha 9$ and $\alpha 3/\alpha 15$ are the consequences of differences in incidence (infection) and/or duration (persistence). It is noteworthy that E5 of HPV16 may influence immune surveillance by down-regulating both HLA class I (10) and HLA class II (23). Thus, it is possible that the observed patterns are partially attributable to differences in E5 activity, given the lack of a definable E5 ORF for $\alpha 3/\alpha 15$ types.

Interestingly, a preliminary examination of HPV types in the $\alpha 7$ species, which include HPV types 18 and 45, showed a similar albeit less pronounced positive association of these types with cervical ectopy compared with the association for $\alpha 9$ species. Whereas this is consistent with the observed age-group prevalences for types of the $\alpha 7$ species (Fig. 1C), it is a surprising result given the strength of the association of these types with adenocarcinoma (glandular cancers) of the cervix (3, 24, 25). That is, our a priori hypothesis was that $\alpha 7$ types would be even more strongly associated with cervical ectopy than $\alpha 9$ types such that the difference in prevalence patterns between $\alpha 7$ and $\alpha 3/\alpha 15$ is more pronounced than the difference between $\alpha 9$ and $\alpha 3/\alpha 15$. Our crude measurements may not be able to detect more subtle distinctions of type-specific “preferences” for different cell types. Another possibility is that this relation of $\alpha 7$ types with adenocarcinoma may reflect an interaction that occurs at a later stage in the carcinogenic pathway, which is consistent with the observed differences in secondary risk factors between squamous and glandular cancers of the cervix (25–27).

Oral contraceptive use has been associated with detection of HPV in some studies but not in others [see meta-analysis by Green et al. (28)]. As pointed out by Green et al. (28), the relationship of HPV

Table 1. The crude association of degree of cervical ectopy with oral contraceptive use (never, former, or current; $P_{\text{trend}} < 0.0005$)

Degree of ectopy	Oral contraceptive use			Total
	Never (reference)	Former	Current	
No squamocolumnar junction (reference)	160 65%	82 33%	4 2%	246
1st and 2nd quartile	97 28%	149 43%	104 30%	350
3rd and 4th quartile	63 18%	148 42%	138 40%	349
Total	320	379	246	945

NOTE: Raw percentages are shown below the number in each cell. This association remained robust after age stratification ($P_{\text{trend}} < 0.0005$).

Table 2. Results of logistic regression models to examine unadjusted and multivariable associations of having an $\alpha 3/\alpha 15$ type (case) versus having an $\alpha 9$ type (control)

	Unadjusted [OR (95% CI)]	Multivariable [OR (95% CI)]
Age (y)		
<25 (reference)	1 (—)	1 (—)
25-34	1.6 (1.1-2.3)	1.6 (1.1-2.3)
35-44	2.3 (1.5-3.5)	2.1 (1.4-3.2)
45-54	2.4 (1.5-4.0)	1.9 (1.1-3.3)
55-64	3.9 (2.3-6.6)	2.5 (1.3-4.8)
65-74	2.2 (1.2-4.1)	1.3 (0.61-2.9)
≥ 75	9.0 (3.0-27)	5.4 (1.6-18)
P_{trend}	<0.0005	0.002
Degree of ectopy		
No squamocolumnar junction (reference)	1 (—)	1 (—)
1st and 2nd quartile	0.52 (0.37-0.73)	0.68 (0.43-1.1)
3rd and 4th quartile	0.38 (0.27-0.53)	0.53 (0.32-0.86)
P_{trend}	<0.0005	0.009
Oral contraceptive use		
Never (reference)	1 (—)	1 (—)
Former	0.76 (0.56-1.0)	0.95 (0.68-1.3)
Current	0.62 (0.45-0.87)	1.0 (0.71-1.5)

DNA detection and oral contraceptive use is complex because of the effect of oral contraceptive use on the degree of cervical ectopy (15) and possibly on cell proliferation and HPV viral expression (29), with the latter effects expected to increase HPV detection. Here, we found that oral contraceptive use modulates, via cervical ectopy, HPV DNA detection in a HPV species-related manner.

There are also interesting parallels between head and neck and lower genital tract HPVs. In both the upper alimentary canal and the genital tract, cancer is caused by carcinogenic HPV types and mainly by HPV16 in the oropharynx (30, 31) and cervix (3–5), respectively. The oral cavity, like the vagina, is composed primarily of squamous epithelium and HPV-induced cancer is very rare. Consistent with data presented in this analysis, there was a greater prevalence of types of the $\alpha 3/\alpha 15$ species than types of the $\alpha 9$ species (32).

In summary, based on the cumulative evidence, we suggest that the physiologic age of the cervix may influence the HPV types detected in specimens collected from the cervical os. Analyses in other population-based studies are needed to confirm this finding. Of note, a previous report in a U.S. population (33) found similar age patterns for “uncharacterized” types, which included and were likely dominated by the common types of the $\alpha 3/\alpha 15$ species (HPV61, HPV71, HPV72, and HPV81), with the age pattern observed for $\alpha 3/\alpha 15$ species in this study. Although primary differences in carcinogenicity have been ascribed to functional differences in E6 and E7 protein activity between HPV types, we suggest that another attribute of carcinogenicity might be the primary interaction with the susceptible cell type, the squamous metaplasia epithelial cells of the cervical transformation zone, where the vast majority of HPV-induced cancer occurs. Future studies are needed to explore differential expression of genes in squamous, squamous metaplastic, and columnar cells to better understand the differences in these phenotypes and how they might influence the natural history of HPV infections. Noncarcinogenic HPV types of the $\alpha 3/\alpha 15$ species may poorly interact with the cancer-susceptible tissue but the biological underpinnings are needed to explain these observations and to rule out artifact or chance. HPV types are genetically closely related, and the HPV genome is relatively small such that laboratory investigations, using functional genetics, may be able to successfully interrogate and explain these observed HPV species-related differences.

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