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Research Article

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HPV Type Attribution in High-Grade Cervical Lesions: Assessing the Potential Benefits of Vaccines in a Population-Based Evaluation in the United States

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Abstract

Background: Two currently available vaccines targeting human papillomavirus (HPV) types 16 and 18 could prevent 70% of cervical cancers and 50% of high-grade cervical lesions. Next-generation vaccines against additional types, such as a candidate 9-valent vaccine against HPV6/11/16/18/31/33/45/52/58, could further reduce HPV-associated disease burden.

Methods: HPV was typed in archived tissues from women ages 21 to 39 years residing in five catchment areas in the United States with cervical intraepithelial neoplasia 2/3 and adenocarcinoma *in situ* (CIN2+) using L1 consensus PCR and type-specific hybridization. Type attribution was estimated using weights to account for lesions with multiple types detected.

Results: From 2008 to 2011, 5,498 of 6,306 (87.2%) specimens obtained from 8,469 women with CIN2+ had valid typing results; HPV DNA was detected in 97.3%. Overall, 50.1% of lesions were attributable to HPV16/18, ranging from 50.3% to 52.4% among those ages 21 to 34 years, and significantly declined in 35 to 39 year-olds (43.5%). HPV16/18 attribution was higher in non-Hispanic whites (56.4%) versus racial/ethnic minorities (range, 41.8%–45.9%; $P < 0.001$). HPV31/33/45/52/58 attribution was 25.0% overall and increased with age ($P < 0.001$). A higher proportion of CIN2+ was attributable to HPV31/33/45/52/58 in non-Hispanic black (29.9%), Hispanic (29.2%), and Asian (33.1%) women compared with non-Hispanic whites (22.8%; $P < 0.001$).

Conclusions: Overall, 75% of lesions were attributable to 7 oncogenic HPV types: 50% to HPV16/18 and 25% to HPV31/33/45/52/58. HPV16/18 had the largest attributable fraction in CIN2+ across all subpopulations, although to a lesser extent in older women and racial/ethnic minorities.

Impact: Vaccines targeting additional oncogenic HPV types could prevent more high-grade cervical lesions, especially among racial/ethnic minorities. *Cancer Epidemiol Biomarkers Prev; 24(2); 393–9. ©2014 AACR.*

Introduction

Of the over 150 human papillomavirus (HPV) types identified to date, 12 are recognized human carcinogens (1) and are causally associated with anogenital and oropharyngeal cancers, of which cervical cancer is the most common (2, 3). Although persistent infection with any carcinogenic (high-risk) HPV type can cause cancer, the relative carcinogenicity varies among individual high-risk types (4). Epidemiologic and laboratory studies have identified HPV 16 to be the most potent carcinogen among all high-risk types, and HPV 16 and 18 together account for 70% of cervical cancers and a large proportion of other HPV-associated cancers worldwide (5, 6). Vaccines directed against HPV 16 and 18 have been commercially available since 2006 (7), but vaccine coverage rates have remained suboptimal in the United States (8).

Invasive cervical cancer is usually preceded by asymptomatic high-grade cervical lesions that are caused by the same high-risk HPV types attributed to cervical cancer (9). Early detection and treatment of high-grade cervical lesions have been the basis for cervical cancer prevention in the United States since the 1950s. As in invasive cancers, HPV 16 and 18 are the most commonly detected types in high-grade cervical lesions; however, there is more heterogeneity in HPV type distribution in high-grade lesions. Studies have shown that HPV 16 and 18 account for a lower proportion of high-grade cervical lesions in Africa and South America compared with North America and Europe and in racial minorities in the United States (10–12).

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Although currently available HPV vaccines have shown some cross-protective efficacy against nonvaccine oncogenic types (13–15), a substantial proportion of high-grade cervical lesions are caused by types against which current vaccines have not shown any degree of efficacy (11, 16, 17). Vaccines that target a wider array of oncogenic HPV types such as a candidate vaccine against 6/11/16/18 and 5 additional oncogenic types (31/33/45/52/58) could provide additional protection against precancerous lesions that require treatment.

The objective of this analysis was to examine carcinogenic HPV type attribution among a sample of U.S. women diagnosed with histologically confirmed cervical intraepithelial neoplasia grade 2 and 3 and adenocarcinoma *in situ* (CIN2+), and to estimate the potential impact of a candidate 9-valent HPV vaccine on CIN2+ lesions among women by age and race/ethnicity.

Materials and Methods

System design/population

The data used in this analysis are from the HPV-IMPACT project. HPV-IMPACT was established in 2008 as a collaboration between the Centers for Disease Control and Prevention (CDC) and 5 sites in the Emerging Infections Program Network to monitor the impact of HPV vaccines on CIN2+ through population-based laboratory surveillance (18). Catchment areas include eight contiguous cities in northwest Alameda County, California; New Haven County, Connecticut; Monroe County, New York; Davidson County, Tennessee; and a contiguous region of Washington and Multnomah Counties, Oregon which includes the city of Portland. The total population of females ages 18 years and older in participating sites ranges from about 230,000 to 330,000 according to the 2010 U.S. Census data. Participating sites and CDC received institutional review board approval or exemption, as appropriate for compliance with local reviews.

Histopathology laboratories serving the catchment areas reported cases of CIN2+ diagnosed in adult (18+ years) female residents of the areas to the HPV-IMPACT monitoring system, either directly or through the state cancer registry. Cases were ascertained with classification systems and nomenclature for high-grade cervical lesions that were in use during the time period. Diagnoses were then grouped by histologic grade into CIN2, CIN2/3 (grade not discriminated), CIN3, and adenocarcinoma *in situ* (AIS) +/− CIN. Reports were de-duplicated within and between reporting laboratories. Date of birth, race/ethnicity, health insurance status, and clinical and HPV vaccination history were collected where available.

Specimen selection and processing

From 2008 to 2011, archived histopathology specimens were obtained for 18- to 39-year-old women reported to the 5 HPV-IMPACT sites. In 2008 and 2009, laboratories were asked to submit specimens on all reported cases, but only a representative sample of specimens (randomly sampled within each CIN diagnosis stratum) was requested from laboratories in 2010 and 2011 to reduce costs. Overall, specimens were obtained for approximately 75% of women reported in the 4-year time period. One block representative of the histologic lesion with the highest grade diagnosis was selected and processed for type-specific HPV DNA testing using a standard protocol as previously described (18). Histologic review of the hematoxylin and eosin (H&E)-stained sections surrounding those used for HPV typing was performed to verify that the high-grade lesion was not lost during recutting. The small size of these lesions increases the likelihood that representative material may not be present in re-cut tissue. Approximately 12% of recut material did not have residual high-grade disease and these were not extracted and tested for HPV.

Laboratory procedures

DNA was extracted and purified either manually with DNeasy (Qiagen) or automated with a Chemagic MSM1 extractor (Perkin Elmer). Both methods were comparable in yield after optimization for FFPE tissues (19). Extracts from all samples were tested with the Linear Array HPV Genotyping Assay (LA; Roche Diagnostics). If HPV52 status was ambiguous due to positive results of the XR probe and the cross hybridizing HPV33, 35, or 58, a quantitative PCR assay was performed to verify the presence of type 52 (20). Samples with inadequate or HPV-negative LA results were retested with the INNO-LiPA HPV Genotyping Extra Assay (Innogenetics) as its short PCR target length might be more suitable for low-quality DNA from formalin-fixed paraffin-embedded (FFPE) tissues. Samples negative for both the genomic control probe and HPV in LA and INNO-LiPA were considered inadequate and omitted from further analysis.

Statistical methods

Analysis was restricted to participants ages 18 to 39 years who were diagnosed with CIN2+ from 2008 to 2011 and whose cervical specimens were adequate for DNA typing ($n = 5{,}378$). We calculated the overall and type-specific prevalence of HPV detection. We evaluated HPV type attribution within demographic and 3 histologic strata. For lesions in which multiple HPV types were detected, we calculated individual type attribution using 2 common methods (5, 21). The first method, hierarchical, attributes each lesion to the most oncogenic HPV type among all types detected in a lesion. This method tends to overestimate the contribution of HPV16 and 18 which are considered to have the greatest oncogenic potential. Therefore, we used a second method, relative attribution, which assumes all types in co-infected lesions contribute to that lesion, but to different degrees. This method estimates individual type attribution by applying weights proportional to the frequency with which each type is detected as a single type in lesions by grade, and divides the proportion of each type by the sum proportions in all types detected in the lesion.

Women with CIN2+ were classified into histologic categories of increasing severity: CIN2, CIN2/3 (i.e., grade not specified, combination of both grades), CIN3/AIS. Women with AIS (with or without CIN) were combined with CIN3 due to small numbers. We analyzed the data by age using five age groups: 18 to 20, 21 to 24, 25 to 29, 30 to 34, and 35 to 39. Because of smaller sample size in the subanalysis among those with known vaccination status, we used three age groups: 18 to 24, 25 to 29, and 30 to 39. We used self-reported race/ethnicity to classify women into non-Hispanic white, non-Hispanic black, Hispanic, and Asian groups; all other reported races and combinations were combined into an "other" category, and those without race/ethnic information were indicated as missing. HPV typing data was classified into five categories: HPV16/18, HPV31/33/45/52/58, other high-risk types (HPV35/39/51/56/59/66/68), other types, and HPV negative. Confidence intervals (CI) were calculated using Wald or exact (Clopper–Pearson) test for the binomial proportion. We used the Pearson X^2 test for independence to evaluate associations between HPV types and demographic characteristics overall and by

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histologic grade. Two-sided statistical tests were considered significant at the alpha level of 0.05.

We used a log binomial regression model to examine independent correlates of HPV31/33/45/52/58 infection (vs. other HPV type infection) among 18- to 39-year-old women diagnosed with CIN2+. Associations were considered significant if the *P* value was <0.05 and those variables were retained in the main effects model. Confounding was assessed to ensure that no parameter estimate of significant variables changed by ≥30%. All pairwise interactions in the final model were examined, and were considered significant if the *P* value for the likelihood ratio test for the interaction term was <0.05.

Results

From 2008 to 2011, a total of 8,490 women ages 18 to 39 years diagnosed with noninvasive CIN2+ were reported to the HPV-IMPACT monitoring system. Archived specimens were retrieved for 6,177 (72.8%) of diagnosed women, 5,401 (87.4%) of which were histologically adequate and underwent DNA testing. HPV DNA results were obtained for 5,378 (99.6%) of those tested.

Table 1 shows selected characteristics for women with HPV DNA typing results and those for whom typing data were not available. Among women with typing results, 59.8% were ages 21 to 29 years (median age, 27 years); 17.0% were non-Hispanic black, 14.0% were Hispanic, and 4.5% were Asian. Of those with known insurance status, over 90% had either private (64.2%) or public (27.9%) insurance. HPV vaccination status was unknown in over 50% of women with and without typing results.

Table 1. Selected characteristics among women diagnosed with CIN2+ by HPV DNA typing status

	HPV typing data available	
	Yes	No
	n (%)	*n* (%)
Total	5,378 (63.3)	3,112 (36.7)
Diagnosis		
CIN2	2,678 (49.8)	1,510 (48.5)
CIN2/3	870 (16.2)	543 (17.4)
CIN3/AIS	1,830 (34.0)	1,059 (34.0)
Age group, years[a]		
18–20	325 (6.0)	152 (4.9)
21–29	3,218 (59.8)	1,821 (58.5)
30–39	1,835 (34.1)	1,139 (36.6)
Race/ethnicity[a]		
NH white	2,881 (62.0)	1,432 (58.7)
NH black	792 (17.0)	411 (16.8)
Hispanic	651 (14.0)	404 (16.6)
Asian	207 (4.5)	89 (3.6)
Other	117 (2.5)	105 (4.3)
Unknown	730	671
Insurance[a]		
Private	3,009 (64.2)	1,578 (61.1)
Public	1308 (27.9)	798 (30.9)
Uninsured	164 (3.5)	100 (3.9)
Other	204 (4.4)	105 (4.1)
Unknown	693	531
Vaccine status[a]		
Vaccinated	961 (36.2)	383 (32.8)
Not vaccinated	1,691 (63.8)	785 (67.2)
Unknown	2,726	1,944

Abbreviations: CIN2+, cervical intraepithelial neoplasia grades 2, 2/3 (grade not discriminated), 3, and adenocarcinoma *in situ* (AIS); NH, non-Hispanic.
[a]$P < 0.05$.

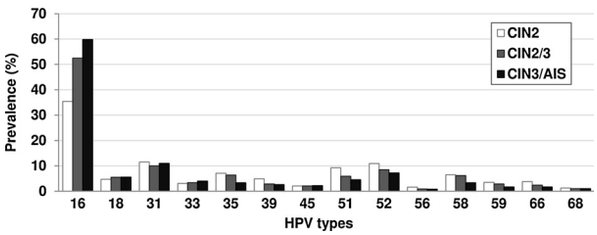

Figure 1.
Type-specific prevalence of high-risk HPV among women with CIN2+, stratified by histologic grade, HPV-IMPACT, 2008–2011. CIN2+, cervical intraepithelial neoplasia grades 2, 2/3 (grade not discriminated), 3, and adenocarcinoma *in situ* (AIS).

HPV detection

Overall, 4,104 (78.5%) of specimens with adequate DNA results had a single HPV type detected, ranging from 76.4% of CIN2 lesions to 82.4% of CIN3/AIS ($P < 0.001$). Among CIN2+ lesions in which multiple types were detected, the majority included only 2 types (78.4%), and less than 60 (5.3%) had 4 or more up to a maximum of 8 types. HPV 16 was the most frequently detected type in all histologic grades, ranging from 35.4% in CIN2 to 59.8% in CIN3/AIS lesions (Fig. 1).

High-risk HPV type attribution

There was no significant difference in HPV type attribution as estimated by the hierarchical and proportional attribution methods, likely due to the high proportion of single type lesions in all histologic grades. Proportional attribution by histologic grade is shown in Fig. 2. Using the proportional attribution method, 49.3% (95% CI, 48.0–50.7) of CIN2+ lesions were attributable to HPV16/18 and 25.2% (95% CI, 24.1–26.4) to the 5 additional types targeted by the candidate 9-valent vaccine, HPV31/33/45/52/58 (Table 2). Overall, 15.9% (95% CI, 14.9–16.9) of CIN2+ lesions were attributable to high-risk HPV types not targeted by candidate 9-valent vaccine, ranging from 21.7% in CIN2 to 8.8% in CIN3/AIS lesions. HPV16/18 relative attribution increased with increasing severity of lesions from 37.7% in CIN2 to 63.5% in CIN3/AIS, whereas HPV31/33/45/52/58 attribution decreased with increasing severity of lesions from 27.6% in CIN2 to 22.7% in CIN3/AIS. HPV 16/18 attribution to CIN2+ lesions ranged from 42.5% in CT to 52.2% in NY, and HPV HPV31/33/45/52/58 attribution ranged from 22.8% in NY to 27.4% in CT.

HPV type attribution by age

HPV16/18 accounted for the highest proportion of CIN2+ lesions across all age groups (Table 2), but the proportional attribution was not constant by age. Among 18 to 20 year-olds, HPV16/18 attribution was 44.0%. The attribution was similar in women ages 21 to 34 (50.0%–51.6%) but was significantly lower (42.4%) in the oldest age group of 35 to 39 year-olds ($P < 0.001$). Conversely, the proportion of CIN2+ lesions attributable to HPV31/33/45/52/58 increased with age and was significantly higher among women ages 35 to 39 years compared with those

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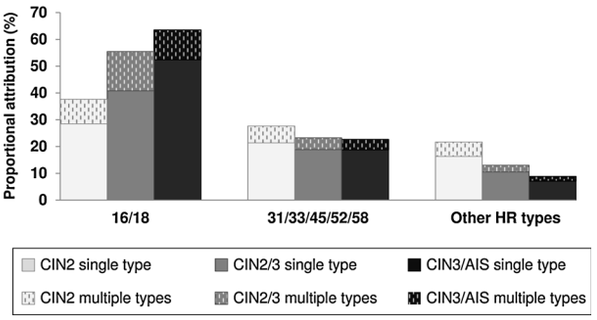

Figure 2.
Proportional attribution of HPV type groups among women with CIN2+, stratified by histologic grade, HPV-IMPACT, 2008–2011. CIN2+, cervical intraepithelial neoplasia grades 2, 2/3 (grade not discriminated), 3, and adenocarcinoma *in situ* (AIS); HR, high risk; other HR types, HPV35/39/51/56/59/66/68.

in younger age groups ($P < 0.001$). Within histologic grade strata (CIN2 and CIN3/AIS), HPV16/18 attribution remained highest across all age groups with the exception of women ages 35 to 39 years with a CIN2 lesion (Fig. 3) and was significantly lower among women ages 35 to 39 compared with those in the younger age groups (CIN2 $P = 0.01$, CIN3/AIS $P < 0.001$). The association of older age with higher prevalence of HPV31/33/45/52/58-attributable lesions remained significant after adjusting for covariates (Supplementary Table S1).

HPV type attribution by race/ethnicity

The largest proportion of CIN2+ lesions was attributable to HPV16/18 types across all racial/ethnic groups (Table 2). However, HPV16/18 were responsible for a significantly higher proportion of CIN2+ lesions among non-Hispanic white (54.2%) compared with non-Hispanic black (40.8%), Hispanic (43.2%), and Asian (42.4%) women ($P < 0.001$). As shown in Fig. 4, stratified by histology, HPV16/18 contributed to a significantly higher proportion of CIN2 and CIN3/AIS lesions in non-Hispanic white women compared with all other racial/ethnic groups, and these differences were greater among women with CIN3/AIS ($P < 0.001$). Racial/ethnic differences in HPV16/18 attribution remained significant when stratified by age group, insurance status, and by catchment area (data not shown).

Among women with CIN2+, HPV31/33/45/52/58 attribution was higher in non-Hispanic black (28.5%), Hispanic (29.5%), and Asian (33.4%) women compared with non-Hispanic white women (22.6%; $P < 0.001$). The proportional attribution of HPV31/33/45/52/58 types did not significantly differ by race/

Table 2. HPV type attribution among women diagnosed with CIN2+, stratified by select characteristics

	16/18		31/33/45/52/58		Other high-risk types[a]	
	n	% (95% CI)	*n*	% (95% CI)	*n*	% (95% CI)
Overall	2,654	49.3 (48.0–50.7)	1,358	25.2 (24.1–26.4)	855	15.9 (14.9–16.9)
Diagnosis						
CIN2	1,008	37.7 (35.8–39.5)	740	27.6 (25.9–29.3)	580	21.7 (20.1–23.2)
CIN2/3	483	55.5 (52.2–58.8)	202	23.3 (20.5–26.1)	113	13.0 (10.8–15.3)
CIN3/AIS	1,162	63.5 (61.3–65.7)	415	22.7 (20.8–24.6)	161	8.8 (7.5–10.1)
Age group, years						
18–20	143	44.0 (38.6–49.4)	66	20.4 (16.1–24.8)	70	21.7 (17.2–26.1)
21–24	737	50.0 (47.4–52.5)	343	23.3 (21.1–25.4)	251	17.0 (15.1–18.9)
25–29	900	51.6 (49.3–54.0)	427	24.5 (22.5–26.5)	260	14.9 (13.2–16.6)
30–34	582	50.8 (47.9–53.7)	296	25.8 (23.3–28.3)	174	15.2 (13.1–17.2)
35–39	291	42.4 (38.7–46.1)	225	32.7 (29.2–36.3)	100	14.6 (11.9–17.2)
Race/ethnicity						
NH white	1,563	54.2 (52.4–56.1)	650	22.6 (21.0–24.1)	410	14.2 (13.0–15.5)
NH black	323	40.8 (37.4–44.2)	225	28.5 (25.3–31.6)	165	20.8 (18.0–23.7)
Hispanic	281	43.2 (39.4–47.0)	192	29.5 (26.0–33.0)	110	16.8 (14.0–19.7)
Asian	88	42.4 (35.7–49.2)	69	33.4 (27.0–39.8)	33	15.8 (10.8–20.8)
Other	50	43.1 (34.1–52.1)	39	33.7 (25.2–42.3)	17	14.8 (8.4–21.3)
Unknown	348	47.7 (44.1–51.3)	182	24.9 (21.7–28.0)	120	16.5 (13.8–19.2)
Insurance						
Private	1,535	51.0 (49.2–52.8)	693	23.0 (21.5–24.5)	488	16.2 (14.9–17.6)
Public	594	45.4 (42.8–48.2)	371	28.3 (25.9–30.8)	221	16.9 (14.9–18.9)
Uninsured	86	52.3 (44.6–59.9)	47	28.8 (21.9–35.7)	17	10.6 (5.9–15.3)
Other	86	42.4 (35.6–49.2)	65	31.9 (25.5–38.3)	33	16.0 (11.0–21.0)
Unknown	352	50.9 (47.1–54.6)	182	26.2 (23.0–29.5)	96	13.8 (11.2–16.4)
Catchment area						
California	442	51.8 (48.4–55.1)	225	26.4 (23.4–29.3)	119	14.0 (11.6–16.3)
Connecticut	575	42.5 (39.8–45.1)	372	27.4 (25.1–29.8)	275	20.3 (18.2–22.4)
New York	801	52.2 (49.7–54.7)	350	22.8 (20.7–24.9)	239	15.6 (13.8–17.4)
Oregon	417	50.6 (47.2–54.0)	221	26.8 (23.8–29.8)	110	13.3 (11.0–15.6)
Tennessee	417	51.6 (48.2–55.0)	189	23.4 (20.5–26.3)	112	13.8 (11.5–16.2)
Vaccination status						
Vaccinated	424	44.1 (41.0–47.3)	236	24.6 (21.9–27.3)	186	19.4 (16.9–21.9)
Not vaccinated	826	48.8 (46.5–51.2)	439	26.0 (23.9–28.1)	267	15.8 (14.0–17.5)
Unknown	1,403	51.5 (49.6–53.4)	682	25.0 (23.4–26.6)	402	14.8 (13.4–16.1)

Abbreviations: CIN2+, cervical intraepithelial neoplasia grades 2, 2/3 (grade not discriminated), 3, and adenocarcinoma *in situ* (AIS); NH, non-Hispanic.
[a]Other high-risk types include HPV35/39/51/56/59/66/68.

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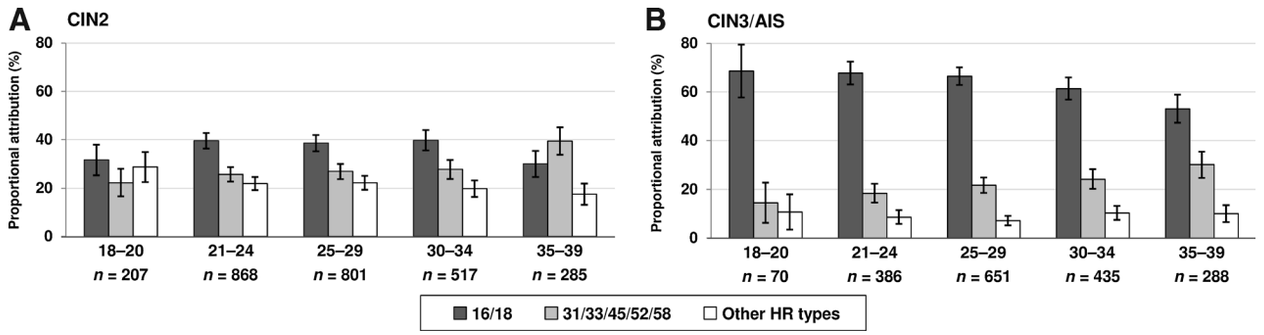

Figure 3.
Proportional attribution of HPV type group among women with CIN2 (A) and CIN3/AIS (B), stratified by age, HPV-IMPACT, 2008–2011. CIN, cervical intraepithelial neoplasia; AIS, adenocarcinoma *in situ*; HR, high risk; other HR types, HPV35/39/51/56/59/66/68.

ethnicity among women with CIN2, but was significantly higher among Asian (35.2%) and non-Hispanic black (29.3%) compared with non-Hispanic white women (19.7%) among those diagnosed with CIN3/AIS ($P < 0.001$). In multivariable analysis, non-Hispanic white women were significantly less likely to have HPV31/33/45/52/58-attributable lesions compared with all other race/ethnic minorities, after adjusting for age at diagnosis, insurance status, and diagnosis grade (Supplementary Table S1).

HPV type attribution by vaccination status

Because vaccination could impact the proportion of CIN2+ attributable to HPV16/18, we repeated the analysis restricted to only unvaccinated women. Among women with known vaccination history, 38.6% (428/1,110) of 18 to 24 year-olds, 68.5% (560/818) of 25 to 29 year-olds, and 97.1% (703/724) of 30 to 39 year-olds were not vaccinated. In these unvaccinated women, HPV16/18 attribution was highest in the youngest age group (54.3%) with a linear decline to 44.5% in the 30 to 39 year-olds ($P = 0.004$). Proportional attribution of HPV16/18 in CIN2+ was significantly lower in women ages 18 to 24 years who had received at least one dose compared with those who were not vaccinated (43.0% vs. 54.3%; $P < 0.001$). There was no significant difference in women ages 25 and older. When we limited our analysis to unvaccinated women, the racial differences in proportion of CIN2+ attributable to HPV16/18 remained; 53.8% in non-Hispanic white, 38.2% in non-Hispanic black, 42.7% in Hispanic, and 46.3% in Asian women ($P < 0.001$). The proportional attribution of HPV31, 33, 45, and other oncogenic types in CIN2+ did not differ by vaccination status among women ages 18 to 24 years (data not shown).

Discussion

In this evaluation of a sample of U.S. women diagnosed with noninvasive CIN2+, HPV DNA was detected in almost all (97%) lesions and over 90% of lesions were associated with at least one high-risk HPV type. HPV16 was the most frequent type detected in all histologic grades overall. HPV16/18 accounted for half of all CIN2+ diagnoses, and the proportion of lesions attributable to HPV16/18 increased with increasing severity of lesion. The additional 5 HPV types in the 9-valent vaccine, HPV31/33/45/52/58, contributed to 25% of CIN2+ lesions in women overall.

We found that HPV type attribution differed by age group. HPV31/33/45/52/58 attribution significantly increased with increasing age, whereas HPV16/18 attribution was lowest in the youngest (18–20 years) and the oldest (35–39 years) age groups of women compared with women ages 21 to 34 years. Positing that the lower proportion of HPV16/18-attributable lesions in the youngest age group is due to vaccination, we examined age group–specific differences in HPV16/18 attribution in a subpopulation of unvaccinated women. As expected, among the unvaccinated women, we found that HPV16/18 attribution was highest in the youngest age group and significantly decreased by age. The

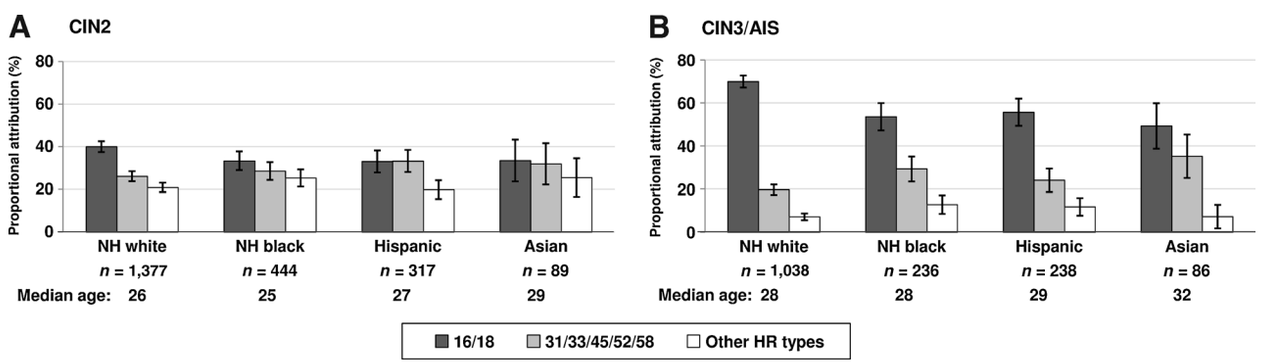

Figure 4.
Proportional attribution of HPV type group among women with CIN2 (A) and CIN3/AIS (B), stratified by race/ethnicity, HPV-IMPACT, 2008–2011. NH, non-Hispanic; CIN, cervical intraepithelial neoplasia; AIS, adenocarcinoma *in situ*; HR, high risk; other HR types, 35/39/51/56/59/66/68.

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relatively lower proportions of HPV16/18-attributable lesions in the older group are congruent with epidemiologic evidence of stronger carcinogenic potential and faster progression of HPV16/18-associated lesions compared with lesions due to other high-risk types, and similar results have been shown in other studies (16, 17, 22, 23).

Our results show a clear predominance of HPV16/18 attribution in CIN2+ lesions among all women in every racial/ethnic group, in agreement with other studies (6, 11, 12, 17, 23–25). However, we found that compared with non-Hispanic white women, HPV31/33/45/52/58 contributes to a significantly higher proportion of CIN2+ in racial minorities. Similar to our findings, a study of U.S. women in New Mexico showed significantly lower proportions of CIN2+ attributable to HPV16/18 in Hispanics compared with non-Hispanic whites (17). It is important to caution against extrapolating results from studies of CIN2+ to make inferences about HPV types detected in cervical cancer because only about 30% of CIN2+ lesions could progress to invasive cancer and those that are attributable to HPV16/18 have a much higher likelihood of progression compared with lesions due to other high-risk types (4, 26, 27). A population-based U.S. study using data from state-based cancer registries found that HPV16/18 are attributable to the same proportion (67%) of invasive cervical cancers in non-Hispanic white and non-Hispanic black women (28). Potential explanations for the observed racial/ethnic differences in HPV type attribution in CIN2+ lesions include differences in the distribution and prevalence of HPV infection, sexual networks and behaviors, host susceptibility, and cervical cancer screening and treatment access and utilization.

CIN2+ lesions, detected through routine screening, account for a large proportion of HPV-associated cervical disease in countries with widespread screening such as the United States. Frequent and repeated screening with the Papanicolau (Pap) test to detect and treat CIN2+ lesions has been successful in substantially reducing the burden of cervical cancer. However, this strategy is resource-intensive and can result in overtreatment of lesions, many of which would have resolved without intervention. More recently, the availability of DNA-based tests that can detect individual or groups of HPV types has led to re-evaluation of screening guidelines. Current screening recommendations include use of HPV-based tests in conjunction with the Pap test in women ages 30 years and older and for follow-up of women with certain abnormalities detected by the Pap test (29). Future screening algorithms that use type-specific HPV detection as the basis for screening or triage are being evaluated. A better understanding of HPV type attribution in CIN2+ lesions and the proportion of lesions that could be prevented by current and candidate vaccines can be important in guiding cervical cancer screening efforts in the era of vaccination and molecular-based testing.

Our study has some limitations. Although we used standard codes and terminology to ascertain and classify lesions, the histology diagnoses were not based on expert pathology consensus review. Interlaboratory variation in diagnosing cervical precancer is recognized to be particularly problematic for CIN2 lesions. In addition, because of heterogeneity within lesions, the residual histology may not match that in the original slides. However, all specimens underwent histologic review by a pathologist (E.R. Unger) and only those with residual high-grade lesions were tested. Refusal by some reporting labs to supply archived tissue may have also introduced bias. To minimize this selection bias, attempts were made to obtain either all or a representative sample of specimens from labs reporting >80% of cases in each catchment. Differences in pathologic diagnosis, especially in CIN2 lesions, is another potential source of bias. Although the inherent heterogeneity in CIN2+ lesions could not be addressed, we conducted stratified analyses by histologic grade. We were unable to obtain race/ethnicity on 13.6% of women, but given the small proportion, any bias resulting from missing data would likely be negligible. Finally, because our data were collected after HPV vaccine introduction, our results were likely influenced by vaccination in age-eligible women in this population. Since HPV vaccine introduction in 2006, vaccination has been recommended through age 26 years for those not vaccinated when they were younger (30). Although there are no data in the exact age groups used in this analysis, the National Health Interview Survey found that 29.5% of women ages 19 to 26 years reported receipt of $\geq$1dose of HPV vaccine in 2011 (31). Any impact in this population would be limited to the youngest women, because vaccination is not recommended in women over age 26 years. Even if some older women were vaccinated in this population, they were likely to have been vaccinated after exposure to vaccine types. Although vaccination history was not available for the entire population, we were able to evaluate type attribution by age and by race/ethnicity among a subgroup of vaccine-eligible women whose records indicated that they were not vaccinated.

In summary, 50% of all CIN2+ lesions diagnosed in this population were attributable to HPV16/18 types in the current vaccines, and another 25% were due to HPV31/33/45/52/58 in the candidate 9-valent vaccine. Although our results indicate lower proportions of CIN2+ lesions attributable to HPV16/18 in racial/ethnic minorities compared with non-Hispanic white women, our finding that HPV16/18 attribution was uniformly highest in all age and racial/ethnic strata strongly supports the benefit of currently available HPV vaccines in the entire U.S. population. HPV vaccines directed against a wider array of high-risk HPV types could prevent additional HPV-associated disease and reduce racial/ethnic differences in high-grade cervical lesions.

Disclosure of Potential Conflicts of Interest

L.M. Niccolai is a consultant/advisory board member for Merck. No potential conflicts of interest were disclosed by the other authors.

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the funding agency.

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