Human Papillomavirus Genotype Distributions: Implications for Vaccination and Cancer Screening in the United States

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Background Limited data are available describing human papillomavirus (HPV) genotype distributions in cervical cancer in the United States. Such studies are needed to predict how HPV vaccination and HPV-based screening will influence cervical cancer prevention.

Methods We used the New Mexico Surveillance, Epidemiology, and End Results Registry to ascertain cases of in situ (n = 1213) and invasive (n = 808) cervical cancer diagnosed during 1985–1999 and 1980–1999, respectively, in the state of New Mexico. HPV genotyping was performed using two polymerase chain reaction–based methods on paraffin-embedded tissues from in situ and invasive cancers and on cervical Papanicolaou test specimen from control subjects (ie, women aged 18–40 years attending clinics for routine cervical screening [n = 4007]). Relative risks for cervical cancer were estimated, and factors associated with age at cancer diagnosis and the prevalence of HPV genotypes in cancers were examined.

Results The most common HPV genotypes detected in invasive cancers were HPV type 16 (HPV16, 53.2%), HPV18 (13.1%), and HPV45 (6.1%) and those in in situ cancers were HPV16 (56.3%), HPV31 (12.6%), and HPV33 (8.0%). Invasive cancer case subjects who were positive for HPV16 or 18 were diagnosed at younger ages than those who were positive for other carcinogenic HPV genotypes (mean age at diagnosis: 48.1 [95% confidence interval {CI} = 46.6 to 49.6 years], 45.9 [95% CI = 42.9 to 49.0 years], and 52.3 years [95% CI = 50.0 to 54.6 years], respectively). The proportion of HPV16-positive in situ and invasive cancers, but not of HPV18-positive cancers, declined with more recent calendar year of diagnosis, whereas the proportion positive for carcinogenic HPV genotypes other than HPV18 increased.

Conclusions HPV16 and 18 caused the majority of invasive cervical cancer in this population sample of US women, but the proportion attributable to HPV16 declined over the last 20 years. The age at diagnosis of HPV16- and HPV18-related cancers was 5 years earlier than that of cancers caused by carcinogenic HPV genotypes other than HPV16 and 18, suggesting that the age at initiation of cervical screening could be delayed in HPV-vaccinated populations.

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Two new approaches for the prevention of cervical cancer have emerged over the past decade: vaccination for the primary prevention of human papillomavirus (HPV) infection in adolescent girls and the use of methods to detect infection with carcinogenic HPV types, which allow secondary prevention via the identification and treatment of precancerous cervical lesions and early-stage cervical cancers. Population-based studies of HPV genotype prevalence are needed to predict how these two approaches might influence cervical cancer prevention and how prophylactic HPV vaccination of young women could affect the secondary prevention of cervical cancer. However, there have been relatively few studies of the distribution of HPV genotypes in the United States. A recent survey from the National Health and Nutrition Examination Survey examined the prevalence of HPV genotypes in a population of women by using self-collected vaginal swab specimens (1), which provided little insight to the HPV genotypes found in precancer and cancer.

Only two moderately large US studies of the HPV genotypes detected in cervical cancer have been reported. Schwartz et al. (2)

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CONTEXT AND CAVEATS

Prior knowledge

Vaccination of adolescent girls for the primary prevention of human papillomavirus (HPV) infection and methods to detect infection with carcinogenic HPV types have emerged as approaches for the prevention of cervical cancer. Population-based studies of HPV genotype prevalence are needed to predict how these approaches might influence cervical cancer prevention.

Study design

A case–control study of Hispanic and non-Hispanic white women in New Mexico to describe cervical cancer risk by HPV genotype, species, and risk groups.

Contribution

HPV16 and 18 caused the majority of cervical cancer in this population sample of US women. However, the proportion of HPV16-positive cancers declined over the last 20 years, and age at diagnosis of HPV16- and HPV18-related cancers was 5 years earlier than that of cancers caused by other carcinogenic HPV genotypes.

Implications

The earlier age at diagnosis of HPV16- and HPV18-related cancers suggests that the age at initiation of cervical screening could be delayed in populations that are vaccinated against those HPV types.

Limitations

The findings may not be generalizable to other populations. Relationships between outcomes and time were inferred. Misattribution of some cases of cervical disease to HPV16 and 18 was likely.

From the Editors

examined HPV genotypes in paraffin-embedded tissues from 399 invasive cervical cancer cases that were diagnosed in Washington State between 1986 and 1997, including 275 squamous cell carcinomas and 87 adenocarcinomas. Among the cancers in which HPV was detected, an unusually high percentage of squamous cell carcinomas (87%) and adenocarcinomas (86%) were positive for HPV type 16 (HPV16) and/or HPV18 compared with an international series (3,4). Burger et al. (5) evaluated HPV genotypes in fresh-frozen tissues from 291 cancer cases using HPV genotype–specific polymerase chain reaction (PCR) primers specific for HPV16, 18, and 6 in addition to a broad-spectrum HPV detection method, which led to a detection bias for those HPV genotypes. However, neither study included precancerous lesions, which are the target of cervical cancer screening programs.

To address this lack of data, we examined HPV genotypes in precancerous cervical lesions (carcinoma in situ [CIS] and adenocarcinoma in situ [AIS]) and invasive cervical cancers diagnosed in the state of New Mexico. We compared the HPV genotype distribution in these cases with that in a control sample of women aged 18–40 years who were attending clinics in New Mexico for routine cervical screening. This control sample was chosen to represent the HPV genotype distribution in the source population for the cervical cancer cases. Our study design contrasts with the typical age-matched case–control study design in which, in the case of cervical cancer, older women would be selected as control subjects. Older women who test positive for HPV represent an unusual comparison group in that they are more likely to have persistent prevalent HPV infection (6), which is a central risk factor for cervical precancer or cancer, and therefore are not representative of the population at risk.

Subjects and Methods

Selection of Case and Control Subjects

The aim of this study was to provide a description of cervical cancer risk by HPV genotype, species, and risk groups. An additional aim, which is the subject of a separate report, included analyses of immunogenetic risk factors. This additional aim prohibited the inclusion of racial groups in which genetic studies were not feasible or for which sample sizes with adequate statistical power could not be achieved. The study was therefore restricted to Hispanic and non-Hispanic white women, who together represent approximately 90% of the female population of New Mexico. These studies were approved by the University of New Mexico Human Research Review Committee.

All cases of invasive cervical cancer diagnosed throughout the state of New Mexico from January 1, 1980, through September 30, 1999, and all cases of CIS or AIS (ie, CIS/AIS) diagnosed at the University of New Mexico Hospital (UNMH) and Lovelace Medical Center (both in Albuquerque, NM) from January 1, 1985, through December 31, 1999, were identified by the New Mexico Tumor Registry (NMTR). The NMTR is a member of the Surveillance, Epidemiology, and End Results (SEER) program of the US National Cancer Institute and has recorded the incidence of cancer in New Mexico since 1969. The NMTR assigns ethnicity based on information from multiple sources, including medical records, surname, Indian Health Service records, and death certificates. We validated the registry's coding of Hispanic and non-Hispanic white ethnicity using data obtained from a case–control study of lung cancer in New Mexico, as previously described (7). Approximately 40% of all CIS/AIS cases diagnosed in New Mexico during this time period (January 1, 1985, through December 31, 1999) were diagnosed at UNMH or the Lovelace Medical Center. Mortality data for cases of cervical cancer included in this study were assessed through the end of November 2006.

Of 1429 cases of invasive cervical cancer diagnosed in Hispanic and non-Hispanic white women throughout New Mexico from 1980 through 1999, a total of 808 women (57%) had paraffin-embedded tissue blocks available for analysis. However, as a result of routine practice, many diagnostic tissues had been discarded after 10 years of archival storage, and therefore, only 42% (n = 283) of paraffin-embedded tissues for invasive cancers diagnosed from 1980 through 1989 were obtained. By contrast, 73% (n = 299) of paraffin-embedded tissues for invasive cancers diagnosed from 1990 through 1994 and 66% (n = 226) of those diagnosed from 1995 through 1999 were obtained.

Of 1530 cases of CIS/AIS diagnosed at UNMH and Lovelace Medical Center from 1980 through 1999, we were able to retrieve paraffin-embedded tissue specimens for the majority (79%; n = 1213): 49% (n = 173) of the CIS/AIS diagnosed from 1985 through 1989, 74% (n = 391) of those diagnosed from 1990 through 1994, and 99% (n = 649) of those diagnosed from 1995 through 1999. Paraffin-embedded tissues, corresponding pathology slides, and surgical pathology reports were obtained for the primary diagnostic

specimen for each case using the initial date of diagnosis maintained in the NMTR database. Original pathology slides were reviewed by the study pathologist (N. E. Joste). When multiple paraffin-embedded tissues were present for a single case, the paraffin-embedded tissue used for this analysis was selected in the following order of priority: the original cervical punch biopsy sample, followed by the loop excision specimen, followed by the hysterectomy specimen. The paraffin-embedded tissues selected for this analysis were subjected to a sandwich technique in which we obtained an initial 4-μm tissue section for hematoxylin–eosin (H&E) staining, followed by three 4-μm sections that were collected in separate Eppendorf tubes for use in PCR assays, followed by up to four additional sections for additional testing that might require microdissection, and a final 4-μm section for H&E staining. The final section was reviewed in a blinded fashion by the study pathologist for comparison with the community-based histology diagnosis and to confirm the presence of cervical cancer or lesion. The presence of either a cervical neoplastic lesion or cervical cancer was confirmed in 87% of the samples: 81% of CIS/AIS and 96% of invasive cancers. When either a cervical lesion or cancer was confirmed to be present, there was 94% agreement between the histology diagnosed by the study pathologist and the community-based histology (squamous, adenocarcinoma, adenosquamous, or other): 96% for CIS/AIS and 91% for invasive cancers. All specimens, regardless of the confirmed presence of cervical cancer or lesion, were used in this analysis.

Control samples consisted of fresh cervical swab specimens obtained from Hispanic and non-Hispanic white women 18–40 years of age who attended the UNMH and Lovelace Medical Center for routine cervical screening. These women were enrolled from the clinic referral base for the CIS/AIS case subjects to represent the HPV exposure distribution in the source population of the cervical precancer and cancer case subjects. More than 4000 control subjects were enrolled in this study from July 1, 1996, through May 31, 2000, after providing written informed consent. Women who self-reported their ethnicity as non-Hispanic white or Hispanic were invited to participate in this study. Enrollment exclusion criteria for control subjects included a history of high-grade squamous intraepithelial lesions (HSILs) or cancer, hysterectomy, a history of cervical excision or ablative treatments, and a diagnosis of any cervical abnormality during the previous year. Fourteen control subjects who had a biopsy-confirmed diagnosis of CIS/AIS during the enrollment period were reclassified as case subjects. At the enrollment visit, 90.9% of control subjects had a normal cytology diagnosis, 6.6% had a diagnosis of atypical squamous cells of undetermined significance, 1.8% had a diagnosis of low-grade squamous intraepithelial lesion, 0.5% had a diagnosis of HSIL, and 0.3% had a diagnosis of atypical glandular cells of undetermined significance.

Laboratory Procedures

Control specimens were collected in specimen transport medium (1 mL; Digene Corp., Gaithersburg, MD) and frozen on the same day of collection at −85°C. The frozen samples were thawed, digestion solution (30 μL of 20 mg/mL proteinase K, 10% laureth-12, 20 mM Tris, and 1 mM EDTA [pH 8.5]) was added to each specimen, and the mixtures were incubated at 60°C for 1 hour. A 300-μL aliquot of the digested material was added to 1.0 mL of absolute ethanol containing 0.5 M ammonium acetate, and the mixture was stored at −20°C overnight to allow the DNA to precipitate and then centrifuged for 30 minutes at 13 000*g*. The supernatant was discarded immediately, and the crude DNA pellet was dried overnight at room temperature. The pellet was suspended in 150 μL of 20 mM Tris and 1 mM EDTA (pH 8.5). A microcentrifuge tube cap lock (GeneMate; ISC BioExpress, Kaysville, UT) was placed on each microcentrifuge tube, and the tubes were incubated for 15 minutes at 95°C to inactivate the proteinase K. The resulting crude DNA extracts were either used immediately for HPV genotyping or stored at −85°C. Crude DNA was obtained from paraffin-embedded tissue sections as previously described (8).

HPV genotyping was performed using two different PCR-based strategies. We initially used the PGMY09/11 line blot assay (LBA) on all samples, a PCR-based assay system that amplifies a broad spectrum of HPV genotypes by targeting a 450-bp fragment within the L1 open reading frame (ORF) of the HPV genome and includes coamplification of an internal 248-bp fragment of the human β-globin gene to assess specimen adequacy (9,10). It is generally accepted that carcinogenic HPV genotypes are necessary causative agents of invasive cervical cancer (11). Therefore, to overcome misclassification of the HPV genotype resulting from potentially degraded DNA in aging archival paraffin-embedded tissues (ie, those archived for >5 years), we also used a second PCR-based strategy, the short fragment SPF10 LiPA25 system (version 1 assay; Labo Biomedical Products bv, Rijswijk, the Netherlands), which amplifies a 65-bp fragment within the L1 ORF (12,13) in supplementary analyses of the following subsets of paraffin-embedded case materials and control specimens: 1) all β-globin negative specimens, 2) all HPV (PGMY09/11 LBA)–negative CIS/AIS and invasive cancer specimens and an equal number of randomly selected HPV (PGMY09/11 LBA)–positive specimens, and 3) a randomly selected sample of 50 HPV (PGMY09/11 LBA)–negative and 50 HPV (PGMY09/11 LBA)–positive control specimens. We used the results of the SPF10 LiPA25 system to assign the HPV genotype for HPV (PGMY09/11 LBA)–negative CIS/AIS and invasive cancer specimens and for all β-globin–negative specimens. For all other specimens, the result of the PGMY09/11 LBA system was used to assign the HPV genotype.

The PGMY09/11 LBA and linear array (LA), a commercialized version of the LBA, and SPF10 LiPA25 assays have demonstrated highly concordant results previously in cervical scrape specimens (14–16). No difference in the overall grouped detection of carcinogenic HPV by the SPF10 LiPA25 and PGMY09/11 LA methods (35.3% vs 35.9%, respectively, $P = .5$) has been observed, with a 91.8% overall agreement and a kappa of 0.82 (16). PGMY09/11 and SPF10 have differences in analytic sensitivity for a few individual HPV genotypes and PGMY09/11 LA has been shown to have an increased sensitivity for detection of HPV coinfections (14,16). Thus, SPF10 primer systems have not been shown to be generally more sensitive for HPV detection than the PGMY09/11 primer system. Rather because SPF10 primers target a smaller HPV L1 fragment, they have potential additional value if DNA is partially degraded into smaller fragments as a result of fixation or paraffin embedding. DNA degradation in paraffin-embedded

tissues does not appear to have differential specificity for given DNA sequences. However, degradation can be directly related to the duration of fixation (eg, time held in neutral buffered formalin) and the time since the specimen was originally embedded (8). With regard to cervical scrape specimens, DNA degradation is minimal to nonexistent particularly when cervical specimens are collected in specimen transport medium and properly stored.

In this study, the SPF10 LiPA25 assay did not demonstrate a detection bias for any particular HPV genotype in paraffin-embedded tissues or cervical scrapes. Our goal in combining test results was to maximize the detection of HPV genotypes, rather than to evaluate the performance of any one test. Among the samples that tested positive for HPV by PGMY09/11 LBA and were retested by SPF10 LiPA25, the crude percent agreement between the two assays was at least 95% for most HPV genotypes (Supplementary Table 1, available online). Importantly, there were no substantial differences between the two assays in the distribution of HPV genotypes in cancers (Supplementary Table 2, available online). Thus, combining the results did not influence the relative importance of HPV genotypes and also demonstrated that the results were not strongly influenced by the HPV genotyping system.

The PGMY09/11 PCR amplimers for all case and control specimens were subjected to reverse line blot hybridization (9,10) for detection of 27 HPV genotypes (HPV6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51–59, 66, 68, 73 [PAP238a], 82 [W13b], 83 [Pap291], and 84 [PAP155]). All control samples and all HPV DNA–negative case samples were also tested by reverse line blot hybridization for an additional 11 noncarcinogenic HPV genotypes (HPV61, 62, 64, 67, 69–72, 81, 82 variant [IS39], and 89 [CP6108]).

For the SPF10 LiPA25 PCR amplifications, the presence of HPV DNA amplimers was examined by hybridization to a mixture of HPV probes that recognize a broad range of at least 54 high-risk, low-risk, and possible high-risk HPV genotypes in a DNA–enzyme immunoassay microtiter plate format, as described previously (12). Resultant amplimers were subjected to reverse line blot hybridization for detection of individual HPV genotypes. The SPF10 LiPA25 assay includes specific probes for the detection of 25 HPV genotypes (HPV6, 11, 16, 18, 31, 33–35, 39, 40, 42–45, 51–54, 56, 58, 59, 66, 68/73, 70, and 74).

Statistical Analysis

For the purpose of computing relative risks (RRs), we considered three different classifications of HPV: 1) HPV genotypes, 2) HPV species, and 3) a categorization based on an a priori risk of cervical cancer. We considered HPV species in addition to HPV genotypes because the species classification (17) has been shown to predict the natural history and carcinogenicity of HPV genotypes within the species (18), and it corroborates much of the recent risk assessments defined by cross-sectional data from case–control studies and case series of cervical cancer (3). In addition, phylogenetic classifications predict some viral patterns, such as the cross-protective effects of the L1 vaccine against HPV16 and 18 (19,20), HPV type representation in precancerous lesions (vs cancer) (4), and the likelihood of causing cytological changes (21), better than risk classifications. We used the following alpha species classification (17): alpha-1 (HPV42), alpha-3 (HPV61, 62, 72, 81, 83, 84, 89), alpha-5 (HPV26, 51, 69, 82, 82 variant IS39), alpha-6 (HPV53, 56, 66), alpha-7 (HPV18, 39, 45, 59, 68, 70), alpha-8 (HPV40, 43), alpha-9 (HPV16, 31, 33, 35, 52, 58), alpha-10 (HPV6, 11, 44, 55, 74), alpha-11 (HPV34, 64, 73), alpha-13 (HPV54), and alpha-15 (HPV71).

The a priori risk of cervical cancer (2,4) was classified as follows (highest to lowest risk): 1) positive for HPV16 only; 2) if not positive for HPV16, then positive for HPV18; 3) if not positive for HPV16 or 18, then positive for other carcinogenic genotypes (HPV31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, or 68); 4) if not positive for HPV16, 18, or any other carcinogenic HPV genotype, then positive for any remaining HPV genotype; and 5) HPV negative. It should be noted that this is a hierarchical classification that results in mutually exclusive categories. Specifically, the number of case and control subjects classified as HPV18 positive (category 2) will be less than the total number positive for HPV18 because category 2 excludes those that are also positive for HPV16. This mutually exclusive categorization is in contrast with the genotype and species classifications, in which subjects could be included in more than one category if they were infected with multiple HPV genotypes.

We estimated the relative risk as the case–control odds ratio and computed odds ratios separately for CIS/AIS and invasive cancers for the individual HPV genotypes or species using multivariable logistic regression. Indicator variables were included for each HPV genotype (or species), which allowed us to adjust for the presence of other HPV genotypes or species when estimating the case–control odds ratio. HPV genotypes 26, 55, 57, 61, 62, 64, 67, 69–72, 81, 82, 82 variant IS39, 83, 84, and 89 were combined into a single "other" category because of low frequency (0 or 1 instance) in either CIS/AIS or invasive cancers. All odds ratios were adjusted for ethnicity (Hispanic vs non-Hispanic white).

We used subjects who were HPV16 positive as the referent category rather than HPV-negative subjects for several reasons. First, it is now generally accepted that there is virtually no risk of cervical precancer or cancer for women who are not infected with HPV (11). Cases of CIS/AIS and invasive cervical cancer that test negative for HPV are, therefore, most likely the result of assay error. Specifically, in the case of archival tissues, HPV-negative cervical cancers may represent a failure to amplify the HPV DNA target due to degradation of the sample following fixation in neutral-buffered formalin. Second, we used HPV16-positive subjects as the referent category because HVP16 generally has the highest prevalence in women with normal cytology and in women with CIS/AIS or invasive cervical cancer (3). In addition, there were sufficient numbers of HPV16-positive subjects in all subgroups of case subjects, which allowed us to use this group as a common referent category throughout our analyses. Another benefit of using HPV16-positive subjects as the referent category is that we need only assume that the relative (vs absolute) frequency of HPV genotypes in the control group is the same as that in the source population of the case subjects. We interpret the case–control odds ratio as the risk of CIS/AIS or invasive cervical cancer for a specific HPV genotype or species relative to the risk for the HPV16-positive group. These case–control odds ratios can also be interpreted as the ratio of two relative risks, each using the unexposed (ie, HPV-negative) category as the referent group.

HPV18 and, to a lesser extent, HPV45 were previously reported to be underrepresented in precancerous lesions of the cervix compared with their prevalence in cancer (4). This observation has direct implications regarding the HPV genotypes that might be preferentially missed by cytology screening. Therefore, we also computed the HPV prevalence ratio (PR) for invasive cancers vs CIS/AIS for each HPV genotype and species and for each category in the hierarchical risk classification. These prevalence ratios are the crude (unadjusted) ratios of the frequency of each HPV type or species in invasive cancer to the corresponding frequency in CIS/AIS. The 95% confidence intervals (CIs) are based on the normal approximation to the binomial distribution.

We investigated the factors associated with age at diagnosis of CIS/AIS and invasive cancer using an analysis of variance model that included HPV hierarchical risk group, ethnicity, histology, stage at diagnosis, and calendar year of diagnosis as predictors. We also investigated the factors associated with the prevalence of HPV genotypes 16 and 18 in the combined sample of CIS/AIS and invasive cancer case subjects using a multinomial regression model that included age at diagnosis, ethnicity, calendar year of diagnosis, histology, and stage of diagnosis as predictors. Our a priori hypothesis was that HPV risk groups might be differentially represented in various subgroups because of a number of factors, including secular trends due to cervical screening, aging in relation to the carcinogenicity of HPV risk groups, and HPV genotype–specific cell interactions. The multinomial regression model allowed us to control for the influence of these factors and to estimate the proportions of HPV risk groups within a subgroup. Importantly, the model would enable us to predict the impact of HPV16/18 vaccination on each subgroup. All statistical analysis was done with SAS version 9.1 software (SAS Institute, Inc., Cary, NC), except for the multinomial regression, which was computed using SUDAAN version 9.0.1 software (Research Triangle Institute, Research Triangle Park, NC). All *P* values are two-sided, and *P* less than .05 was considered statistically significant.

Results

Control subjects were predominantly Hispanic (57.0%), whereas the majority of case subjects were non-Hispanic white (58.9% and 58.3%, for subjects with CIS/AIS and invasive cancer, respectively) (Table 1). The majority of cancers (59.0%) were staged by the SEER program as local invasive (22, 23), with decreasing numbers for more severe stages of cancer. Most CIS/AIS (95.9%) and invasive cancers (81.7%) were diagnosed as having squamous cell histology. Not surprisingly, the fraction of deaths attributable to cervical cancer increased with increasing severity of cancer stage at diagnosis, from 33.9% for local-stage invasive cancer to 71.4% for distant-stage invasive cancer.

The distributions of individual HPV genotypes in specimens from control subject and from CIS/AIS and invasive cancer case subjects are shown in Table 2. The prevalence of HPV in the control group specimens was 38.4%, and the most common HPV genotypes were HPV16 (7.4%), HPV53 (4.9%), and HPV54 (3.5%). Most case specimens of CIS/AIS tested HPV positive (97.1%), and the most common genotypes were HPV16 (56.3%), HPV31 (12.6%), and HPV33 (8.0%). HPV18 was found in only 5.9% of CIS/AIS. Most case specimens of invasive cancer tested HPV positive (91.0%), and the most common genotypes were HPV16 (53.2%), HPV18 (13.1%), and HPV45 (6.1%). HPV31 and 33 were found in 12.6% and 8.0% of CIS/AIS, respectively, but in only 4% of invasive cancers. Among the HPV-positive specimens, multiple genotypes were detected in 687 (44.7%) of the control specimens, 215 (18.2%) of the CIS/AIS, and 62 (8.4%) of the invasive cancers. HPV frequencies using pooled results from the PGMY09/11 and SPF10 LiPA25 assays, which include an additional 11 noncarcinogenic HPV types, are shown in Supplementary Table 3 (available online).

We used HPV16-positive subjects as the referent group when computing the case–control odds ratios and, therefore, interpret the odds ratio as a measure of the risk of CIS/AIS or invasive cancer for women infected with a specific HPV genotype relative to the risk for women exposed to HPV16. These odds ratios can also be interpreted as the ratio of two relative risks, each of which used the HPV-unexposed women as the referent group. For brevity, we refer to the case–control odds ratios as relative risks rather than as relative risk ratios and we have expressed the relative risk as a percentage so that HPV16 positivity had a relative risk of 100% and most of the other HPV genotypes had relative risks lower than 100%. Among HPV33-positive women, the relative risk of CIS/AIS was 101% (95% CI = 62% to 163%), which was essentially equal to that for HPV16-positive women and was consistent with a previous observation that HPV33 exposure posed a statistically significant risk for cervical intraepithelial neoplasia grade 2 or 3 (24). Of the remaining HPV genotypes, only genotypes 31 and 35 had relative risks for CIS/AIS that were statistically significantly greater than 10% (HPV31: RR = 43%, 95% CI = 31% to 60%; HPV35: RR = 41%, 95% CI = 24% to 72%). The greatest risks of invasive cancer relative to HPV16 were observed for HPV18 (RR = 68%, 95% CI = 47% to 100%), HPV33 (RR = 57%, 95% CI = 30% to 107%), HPV45 (RR = 32%, 95% CI = 20% to 53%), and HPV35 (RR = 22%, 95% CI = 11% to 47%).

The case subjects were diagnosed from 1980 through 1999, whereas the control subjects were recruited from 1996 through 2000. Therefore, we also computed relative risks using only those precancers and invasive cancers that were diagnosed during 1995–1999, a 5-year period that had the maximum overlap with the control subject enrollment period. (Supplementary Table 4, available online). The same pattern of relative risks was observed for this restricted sample of cases.

Relative risks of CIS/AIS and invasive cancer by HPV species are shown in Table 3. The HPV types in the alpha-9 group are HPV16, 31, 33, 35, 52, and 58. The alpha-7 group includes HPV18, 39, 45, 59, 68, and 70. The risk of CIS/AIS for HPV genotypes in the alpha-9 species (excluding HPV16) relative to HPV16 was 49% (95% CI = 39% to 62%). No other HPV species had a relative risk of CIS/AIS statistically significantly greater than 5%. The risk of invasive cancer for HPV genotypes in the alpha-9 species (excluding HPV16) relative to HPV16 was 19% (95% CI = 14% to 25%). HPV genotypes in the alpha-7 species had a similar relative risk of invasive cancer (RR = 19%, 95% CI = 15% to 26%). The risks of AIS and of adenocarcinoma for alpha-7 relative to HVP16 were 73% (95% CI = 21% to 258%) and 54% (95%

Table 1. Characteristics of study subjects*

Characteristic	Control subjects (N = 4007) n (%)	CIS/AIS case subjects (N = 1213) n (%)	Invasive cancer case subjects (N = 808) n (%)
Year of diagnosis			
1980–1984	0 (0.0)	0 (0.0)	100 (12.4)
1985–1989	0 (0.0)	173 (14.3)	183 (22.6)
1990–1994	0 (0.0)	391 (32.2)	299 (37.0)
1995–2000	4007 (100.0)	649 (53.5)	226 (28.0)
Age at interview or diagnosis, y			
<20	353 (8.8)	66 (5.4)	1 (0.1)
20–24	1047 (26.1)	238 (19.6)	17 (2.1)
25–29	996 (24.9)	278 (22.9)	60 (7.4)
30–39	1477 (36.9)	405 (33.4)	199 (24.6)
40–49	119 (3.0)	136 (11.2)	170 (21.0)
50–59	0 (0.0)	55 (4.5)	123 (15.2)
60–69	0 (0.0)	23 (1.9)	123 (15.2)
70–79	0 (0.0)	9 (0.7)	77 (9.5)
≥80	0 (0.0)	3 (0.2)	38 (4.7)
Missing	15 (0.4)	0 (0.0)	0 (0.0)
Ethnicity			
Non-Hispanic white	1725 (43.0)	714 (58.9)	471 (58.3)
Hispanic	2282 (57.0)	499 (41.1)	337 (41.7)
Stage at diagnosis†			
In situ	—	1213 (100.0)	0 (0.0)
Local	—	0 (0.0)	477 (59.0)
Regional	—	0 (0.0)	269 (33.3)
Distant	—	0 (0.0)	47 (5.8)
Unknown	—	0 (0.0)	15 (1.9)
Histology			
Squamous	—	1163 (95.9)	660 (81.7)
Adenocarcinoma	—	23 (1.9)	101 (12.5)
Adenosquamous	—	2 (0.2)	21 (2.6)
Other	—	25 (2.1)	26 (3.2)
Vital status as of November 2006‡			
Alive	—	1156 (95.3)	450 (55.7)
From cervical cancer	—	2 (0.2)	180 (22.3)
From other causes	—	55 (4.5)	178 (22.0)
Local-stage invasive cancer deaths			
From cervical cancer	—	—	42 (33.9)
From other causes	—	—	82 (66.1)
Regional-stage invasive cancer deaths			
From cervical cancer	—	—	105 (57.1)
From other causes	—	—	79 (42.9)
Distant-stage invasive cancer deaths			
From cervical cancer	—	—	30 (71.4)
From other causes	—	—	12 (28.6)
Unknown-stage invasive cancer deaths			
From cervical cancer	—	—	3 (37.5)
From other causes	—	—	5 (62.5)

* CIS/AIS = carcinoma in situ or adenocarcinoma in situ; — = not applicable.

† Surveillance, Epidemiology, and End Results historic A cancer staging was used (23).

‡ Control subjects were not followed up for survival.

CI = 29% to 98%), respectively. Much of this risk was attributable to HPV18. When HPV18 was excluded, the risks of AIS and of adenocarcinoma for alpha-7 relative to HPV16 were 6% (95% CI = 1% to 66%) and 7% (95% CI = 2% to 19%), respectively (data not shown).

We next stratified by histologic type (squamous cell carcinoma, adenocarcinoma, or adenosquamous carcinoma) and categorized the case and control subjects into one of five mutually exclusive groups according to their a priori risk of cancer based on the results of the HPV genotyping (Table 4). The relative risks of CIS/AIS were negligible for the HPV-negative and noncarcinogenic HPV groups (RR = 0%, 95% CI = 0% to 0%, and RR = 2%, 95% CI = 2% to 3%, respectively), whereas the carcinogenic HPV (excluding HPV16 and 18) and HPV18-positive groups had relative risks for CIS/AIS that were similar (RR = 30%, 95% CI = 25% to 36% and RR = 35%, 95% CI = 24% to 52%, respectively) but substantially smaller than that for the HPV16-positive referent group (100%). The relative risk of invasive cancer showed a similar

Table 2. Relative risks of CIS/AIS and invasive cervical cancer for individual HPV genotypes expressed as the percent risk relative to HPV16*

HPV type	Control subjects (N = 4007) n (%)	CIS/AIS case subjects (N = 1213) n (%)	Invasive cancer case subjects (N = 808) n (%)	CIS/AIS RR (95% CI)	Invasive cancer RR (95% CI)
16	297 (7.4)	683 (56.3)	430 (53.2)	100 (referent)	100 (referent)
06	80 (2.0)	12 (1.0)	4 (0.5)	1 (0 to 2)	0 (0 to 2)
11†	18 (0.4)	14 (1.2)	1 (0.1)	7 (2 to 21)	0 (0 to 4)
18	91 (2.3)	72 (5.9)	106 (13.1)	14 (9 to 21)	68 (47 to 100)
31	117 (2.9)	153 (12.6)	35 (4.3)	43 (31 to 60)	8 (5 to 14)
33	37 (0.9)	97 (8.0)	32 (4.0)	101 (62 to 163)	57 (30 to 107)
35	39 (1.0)	42 (3.5)	14 (1.7)	41 (24 to 72)	22 (11 to 47)
39	132 (3.3)	20 (1.6)	22 (2.7)	1 (0 to 2)	5 (2 to 9)
40‡,§	23 (0.6)	1 (0.1)	0 (0.0)	0 (0 to 2)	—
42†,‡	48 (1.2)	3 (0.2)	1 (0.1)	0 (0 to 1)	0 (0 to 2)
45	90 (2.2)	37 (3.1)	49 (6.1)	7 (4 to 13)	32 (20 to 53)
51	120 (3.0)	59 (4.9)	9 (1.1)	9 (6 to 15)	2 (1 to 4)
52	118 (2.9)	64 (5.3)	24 (3.0)	11 (7 to 16)	6 (3 to 11)
53	197 (4.9)	28 (2.3)	9 (1.1)	1 (1 to 2)	0 (0 to 1)
54	140 (3.5)	3 (0.2)	2 (0.2)	0 (0 to 0)	0 (0 to 1)
56	96 (2.4)	20 (1.6)	9 (1.1)	2 (1 to 5)	3 (1 to 8)
58	100 (2.5)	66 (5.4)	20 (2.5)	15 (9 to 22)	2 (1 to 5)
59	84 (2.1)	17 (1.4)	15 (1.9)	2 (1 to 5)	7 (3 to 14)
66	91 (2.3)	13 (1.1)	2 (0.2)	1 (0 to 2)	0 (0 to 1)
68/73	86 (2.1)	27 (2.2)	13 (1.6)	3 (2 to 6)	6 (3 to 12)
All other types	646 (16.1)	35 (2.9)	7 (0.9)	0 (0 to 0)	0 (0 to 0)
PCR negative‖	2470 (61.6)	35 (2.9)	73 (9.0)	0 (0 to 0)	0 (0 to 1)

* RRs are given as percentage. CIS/AIS = carcinoma in situ or adenocarcinoma in situ; HPV = human papillomavirus; — = not applicable; RR = relative risk; CI = confidence interval; PCR = polymerase chain reaction.

† Not found as a single genotype infection in invasive cancers.

‡ Not found as a single genotype infection in CIS/AIS.

§ Not found in invasive cancer and was not included in the logistic regression model.

‖ On pathology review, 14 of the 35 CIS/AIS and 8 of the 73 invasive cancers had no tumor present in the final hematoxylin-eosin–stained section.

pattern except that the risk for the HPV18-positive group was equal to that of the HPV16-positive group (RR = 100%, 95% CI = 71% to 140%) and much larger than the relative risk of invasive cancer for the carcinogenic HPV group (excluding HPV16 and 18) (RR = 21%, 95% CI = 17% to 26%). When the analysis was restricted to adenocarcinoma and adenosquamous cell carcinoma, the risks of AIS and adenocarcinoma for the HPV18-positive group (RR = 430%, 95% CI = 168% to 1092%, and RR = 325%, 95% CI = 187% to 561%, respectively) were much greater than the corresponding risks for the HPV16-positive referent group. There were no cases of AIS observed in the carcinogenic HPV (excluding HPV16 and 18)-positive group (RR = 0%, 95% CI = 0% to 13%), and the relative risk of invasive adenocarcinoma for this group of HPV types was only 11% (95% CI = 5% to 23%).

To explore the relationship between invasive cancer and CIS/AIS, we calculated HPV PRs (ie, the prevalence of HPV in invasive cancer relative to that in CIS/AIS) (data not shown). For all histological types combined, the prevalence of HPV18 was much higher in invasive cancers than in CIS/AIS (PR = 2.21, 95% CI = 1.66 to 2.94) and exceeded the prevalence ratio for HPV16 (PR = 0.95, 95% CI = 0.87 to 1.03). All other genotypes had PRs less than 0.75, with the exceptions of the alpha-7 genotypes HPV39 (PR = 1.65, 95% CI = 0.91 to 3.01), HPV45 (PR = 1.99, 95% CI = 1.31 to 3.02), and HPV59 (PR = 1.32, 95% CI = 0.67 to 2.64). As a group, the alpha-7 genotypes had a PR of 1.89 (95% CI = 1.55 to 2.29) and excluding HPV18 did not substantially reduce the prevalence ratio (PR = 1.60, 95% CI = 1.19 to 2.14). The PR for alpha-7 genotypes was 1.83 (95% CI = 1.47 to 2.26) for squamous cell histology and for adenocarcinoma it was 0.65 (95% CI = 0.41 to 1.02).

By use of a multivariable analysis of variance approach, we also explored the determinants of age at diagnosis for CIS/AIS and invasive cancer (Table 5). HPV risk group was statistically significantly associated with the age at diagnosis of invasive cancer ($P < .001$) but not with the age at diagnosis of CIS/AIS ($P = .15$). Invasive cancer case subjects who were positive for HPV16 or 18 were diagnosed at statistically significantly younger mean ages than those who were positive for other carcinogenic HPV genotypes (mean age at diagnosis: 48.1 [95% CI = 46.6 to 49.6], 45.9 [95% CI = 42.9 to 49.0], and 52.3 years [95% CI = 50.0 to 54.6], respectively; $P = .0029$ for HPV16 vs other carcinogenic HPV genotypes and $P = .0013$ for HPV18 vs other carcinogenic HPV genotypes). Locally invasive cancers were diagnosed at a statistically significantly younger mean age than cancers that had presented with regional or distant metastases (mean age at diagnosis: 45.2 [95% CI = 43.8 to 46.6], 55.9 [95% CI = 54.0 to 57.8], and 55.1 [95% CI = 50.6 to 59.6] years, respectively: $P < .001$ for both comparisons).

Finally, we examined how the relative proportions of HPV16-positive, HPV18-positive, and other HPV-positive cases of CIS/AIS and invasive cancer varied by calendar year of diagnosis, age at diagnosis, ethnicity, histology, and stage (Table 6). Remarkably,

Table 3. Relative risks of CIS/AIS and invasive cervical cancer for HPV phylogenetic species expressed as the percent risk relative to HPV16*

HPV species	Control subjects (N = 4007) n (%)	CIS/AIS case subjects (N = 1213) n (%)	Invasive cancer case subjects (N = 808) n (%)	CIS/AIS RR (95% CI)	Invasive cancer RR (95% CI)
HPV16	297 (7.4)	683 (56.3)	430 (53.2)	100 (referent)	100 (referent)
Alpha-1	48 (1.2)	3 (0.2)	1 (0.1)	0 (0 to 1)	0 (0 to 2)
Alpha-3	510 (12.7)	12 (1.0)	4 (0.5)	0 (0 to 0)	0 (0 to 0)
Alpha-4†	1 (0.0)	1 (0.1)	0 (0.0)	59 (2 to >999)	—
Alpha-5	160 (4.0)	74 (6.1)	10 (1.2)	6 (4 to 9)	1 (0 to 2)
Alpha-6	342 (8.5)	60 (4.9)	20 (2.5)	1 (1 to 1)	1 (0 to 1)
Alpha-7	466 (11.6)	152 (12.5)	191 (23.6)	3 (2 to 4)	19 (15 to 26)
Alpha-8†	23 (0.6)	1 (0.1)	0 (0.0)	0 (0 to 1)	—
Alpha-9‡	312 (7.8)	342 (28.2)	100 (12.4)	49 (39 to 62)	19 (14 to 25)
Alpha-10	140 (3.5)	30 (2.5)	6 (0.7)	1 (1 to 2)	0 (0 to 1)
Alpha-11	44 (1.1)	17 (1.4)	7 (0.9)	3 (1 to 7)	5 (2 to 15)
Alpha-13	140 (3.5)	3 (0.2)	2 (0.2)	0 (0 to 0)	0 (0 to 1)
Alpha-15†,§	7 (0.2)	0 (0.0)	0 (0.0)	—	—
PCR negative	2470 (61.6)	35 (2.9)	73 (9.0)	0 (0 to 0)	0 (0 to 0)

* RRs are given as percentage. CIS/AIS = carcinoma in situ or adenocarcinoma in situ; HPV = human papillomavirus; — = not applicable; RR = relative risk; CI = confidence interval; PCR = polymerase chain reaction.

† Not found in invasive cancer and was not included in the logistic regression model.

‡ Excludes HPV16.

§ Not found in CIS/AIS and was not included in the logistic regression model.

the proportion of HPV16-positive cases declined with more recent calendar year of diagnosis, whereas the proportion of cases positive for HPV genotypes other than HPV16 or 18 increased. The proportion of HPV18-positive cases showed no clear trend by calendar year of diagnosis. The proportion of HPV16-positive precancers and cancers diagnosed during 1980–1984 was 68.5% compared with 53% during 1995–2000. The proportions of HPV16- and HPV18-positive cases declined with increasing age at diagnosis, which is consistent with the data presented in Table 5. There was a greater prevalence of HPV16-positive cases diagnosed in non-Hispanic white women than in Hispanic women (57.8% and 51.3%, respectively). The combined proportions of HPV16-positive and HPV18-positive cases was greater in glandular (eg, adenocarcinoma) and adenosquamous histology than in squamous cell histology (71.9% and 63.3%, respectively), which reflects the greater contribution of HPV18 to glandular disease than to squamous cell disease (26.7% and 6.2%, respectively). The proportion of HPV18-positive CIS/AIS cases (5.3%) was lower than the proportions of HPV18-positive local-stage (10.5%), regional-stage (14.5%), or distant-stage (13.5%) invasive cancers.

The proportions of HPV16-related alpha-9 and HPV18-related alpha-7 genotype–positive cases remained relatively constant by calendar period of diagnosis (Table 6). This finding suggests that the shift in more recent years from HPV16 and 18 to other HPV genotypes was attributable to other genotypes in the alpha-9 and alpha-7 phylogenetic species rather than to genotypes in the other phylogenetic species that contain carcinogenic HPV genotypes (ie, alpha-5 and alpha-6). In addition, the fact that the proportion of disease at diagnosis due to the other HPV species increased from 3.6% in women aged 30–39 years to 8.4% in women aged 50–59 years, to 26.7% in women aged 80 years or older suggests that alpha-5 and alpha-6 HPV genotypes not only rarely cause cancer but also are very weak carcinogens that take a prolonged time for precancerous lesions to develop into cancer.

Discussion

We conducted a study of HPV genotype patterns in invasive cervical cancer and its immediate precursor lesion, CIS. To our knowledge, this is the largest study of this kind conducted in a US population. The size of this study permitted us to examine HPV genotype patterns in both squamous cell and glandular cell histology. Our findings have direct implications for past and future patterns of cervical cancer incidence and prevention.

We confirmed that HPV genotypes HPV16, 18, 31, 33, 35, 45, 52, and 58 were associated with a high risk of cervical CIS/AIS. In addition, the greatest risk of developing invasive cervical cancer was among women who were positive for HPV genotypes 16, 18, 33, 35, and 45. For CIS/AIS, the mean age at diagnosis did not differ among cases that were positive for HPV16, 18, or other carcinogenic HPV genotypes. By contrast, we found that HPV16- and HPV18-positive invasive cancers were more prevalent in younger women than invasive cancers related to other carcinogenic non-HPV16/18 genotypes. One possible explanation for this age shift is that there may be a longer sojourn time at each stage of cervical carcinogenesis for these less carcinogenic HPV genotypes. It is also possible that these weaker carcinogens are more dependent on secondary factors, such as smoking (25,26). Alternatively, other carcinogenic HPV genotypes may take longer to establish causal infections because of their lower prevalence in the general population. In support of our findings, two very recent reports demonstrated a younger mean or median age at diagnosis for HPV 16 and 18 cancers (27,28). One of these studies also showed that the integration frequency of various HPV genotypes was strongly associated with age at diagnosis of cancer and presumably with malignant potential (27).

Table 4. Relative risks of CIS/AIS and invasive cervical cancer for HPV risk groups expressed as the percentage risk relative to HPV16*

Histological type and HPV risk group	Control subjects (N = 4007) n (%)	CIS/AIS case subjects (N = 1213) n (%)	Invasive cancer case subjects (N = 808) n (%)	CIS/AIS RR (95% CI)	Invasive cancer RR (95% CI)
All histologic types					
PCR negative	2470 (61.6)	35 (2.9)	73 (9.0)	0 (0 to 0)	0 (0 to 0)
Noncarcinogenic	536 (13.4)	30 (2.5)	16 (2.0)	2 (2 to 3)	2 (1 to 3)
Carcinogenic†	635 (15.8)	409 (33.7)	184 (22.8)	30 (25 to 36)	21 (17 to 26)
HPV18	69 (1.7)	56 (4.6)	105 (13.0)	35 (24 to 52)	100 (71 to 140)
HPV16	297 (7.4)	683 (56.3)	430 (53.2)	100 (referent)	100 (referent)
	Control subjects (N = 4007)	**CIS case subjects (N = 1163)**	**Invasive cancer case subjects (N = 660)**	**CIS**	**Invasive cancer**
	n (%)	**n (%)**	**n (%)**	**RR (95% CI)**	**RR (95% CI)**
Squamous cell					
PCR negative	2470 (6.6)	34 (2.9)	32 (4.8)	0 (0 to 0)	0 (0 to 0)
Noncarcinogenic	536 (13.4)	27 (2.3)	16 (2.4)	2 (1 to 3)	2 (1 to 4)
Carcinogenic†	635 (15.8)	400 (34.4)	164 (24.8)	30 (25 to 36)	21 (16 to 26)
HPV18	69 (1.7)	44 (3.8)	65 (9.8)	28 (19 to 43)	70 (48 to 102)
HPV16	297 (7.4)	658 (56.6)	383 (58.0)	100 (referent)	100 (referent)
	Control subjects (N = 4007)	**AIS case subjects (N = 25)**	**Invasive cancer case subjects (N = 122)**	**AIS**	**Invasive cancer**
	n (%)	**n (%)**	**n (%)**	**RR (95% CI)**	**RR (95% CI)**
Adenocarcinoma or adenosquamous‡					
PCR negative	2470 (61.6)	0 (0.0)	32 (26.2)	0 (0 to 3)	9 (5 to 14)
Noncarcinogenic	536 (13.4)	1 (4.0)	0 (0.0)	5 (0 to 32)	0 (0 to 4)
Carcinogenic†	635 (15.8)	0 (0.0)	11 (9.0)	0 (0 to 13)	11 (5 to 23)
HPV18	69 (1.7)	12 (48.0)	34 (27.9)	430 (168 to 1092)	325 (187 to 561)
HPV16	297 (7.4)	12 (48.0)	45 (36.9)	100 (referent)	100 (referent)

* The human papillomavirus (HPV) risk group is defined as a hierarchical classification: HPV16 > HPV18 > carcinogenic HPV except HPV16 and HPV18 > noncarcinogenic HPV > HPV negative. Note that this is a hierarchical classification and that the number in the HPV18 category excludes those with HPV16 coinfection. RRs are given as percentage. CIS/AIS = carcinoma in situ or adenocarcinoma in situ; RR = relative risk; CI = confidence interval; PCR = polymerase chain reaction.

† Excludes HPV16 and 18.

‡ Due to the small number of cases, relative risks for adenocarcinoma and adenosquamous were not adjusted for race.

Regardless of the reason for the age shift, we suggest that it may be reasonable to consider increasing the age at initiation of cervical screening programs (ie, currently 21 years of age in the United States or within 3 years of beginning sexual activity) in HPV-vaccinated populations for several reasons. First, in unvaccinated populations, the incidence of cervical cancer in women younger than 25 years is extremely rare, approximately two in 100 000 (22). It is reasonable to expect that the rates of cervical cancer in vaccinated women younger than 25 years will be reduced by 50%, to one in 100 000, which is similar to the rate of vaginal cancer (22). By analogy to vaginal cancer, for which screening is not recommended, screening in young, vaccinated women should be discouraged. Second, screening is not currently preventing these rare cases of cancer in younger women (22). Third, the prevention of these rare cases of cervical cancer must be weighed against iatrogenic morbidity due to overtreatment by excision of benign lesions. Unfortunately, aggressive cervical screening in this relatively low-risk reproductive-age population leads to the overdetection of histological changes, such as cervical intraepithelial neoplasia (CIN) grade 2 (CIN2), that often result from recent HPV infections. The diagnosis of CIN2 has been shown to be highly heterogeneous, not reproducible among pathologists (29), and is associated with an intermediate risk of cancer compared with a diagnosis of CIN3 (30). CIN2 diagnoses include occult cervical precancers and, therefore, a CIN2 diagnosis represents a mandatory threshold that triggers ablative or excisional treatment under current clinical management guidelines (31). However, excisional treatment of high-grade lesions has been shown to negatively impact reproductive outcomes by increasing risks of premature rupture of membranes, low–birth weight infants, and preterm delivery (32).

It is also worth noting that the positive predictive values or the posttest probabilities of disease for cytology and HPV testing are expected to decrease in vaccinated populations (33,34) because the primary benefits of screening are related to the detection of HPV16. Because of this decrease and because the cancers caused by carcinogenic HPV genotypes other than HPV16 and 18 represent about one-third of cases currently diagnosed in women younger than 30 years of age, the cost-effectiveness of screening younger women is likely to decrease dramatically (35). Increasing the age at initial screening in vaccinated populations will undoubtedly allow some cases of precancer and cancer to go undiagnosed in younger women. However, as many as 60% of cases (36,37) of invasive cervical cancer diagnosed today are detected, regardless of age, in women who have never received a Papanicolaou test or who

Table 5. Mean age at diagnosis of CIS/AIS and invasive cervical cancer from analysis of variance models*

Characteristic	CIS/AIS N	CIS/AIS Mean (95% CI)	Invasive cancer N	Invasive cancer Mean (95% CI)
All study subjects	1213	32.2 (31.5 to 32.8)	808	49.6 (48.4 to 50.7)
HPV risk group				
HPV negative	35	32.4 (28.8 to 35.9)	73	55.4 (51.7 to 59.2)
Noncarcinogenic	30	36.0 (32.1 to 39.8)	16	53.3 (45.6 to 61.0)
Carcinogenic (excluding 16 and 18)	409	32.8 (31.7 to 33.8)	184	52.3 (50.0 to 54.6)
HPV18 positive	56	32.0 (29.0 to 35.0)	105	45.9 (42.9 to 49.0)
HPV16 positive	683	31.6 (30.8 to 32.4)	430	48.1 (46.6 to 49.6)
		$P = .15$		$P < .001$
Ethnicity				
Hispanic	499	30.5 (29.6 to 31.5)	337	49.3 (47.6 to 50.9)
Non-Hispanic white	714	33.3 (32.5 to 34.1)	471	49.8 (48.4 to 51.2)
		$P < .001$		$P = .65$
Histology				
Squamous cell	1163	32.1 (31.4 to 32.7)	660	49.6 (48.4 to 50.8)
Adenocarcinoma	23	33.2 (28.5 to 37.9)	101	51.5 (48.3 to 54.8)
Adenosquamous	2	34.1 (19.1 to 49.1)	21	45.5 (38.8 to 52.2)
Other	25	35.2 (31.0 to 39.5)	26	44.4 (38.2 to 50.6)
		$P = .50$		$P = .13$
Stage at diagnosis for invasive cancer				
Local		—	477	45.2 (43.8 to 46.6)
Regional		—	269	55.9 (54.0 to 57.8)
Distant		—	47	55.1 (50.6 to 59.6)
Unknown		—	15	56.3 (48.3 to 64.4)
				$P < .001$
Year of diagnosis				
1980–1984	0	—	100	52.3 (49.3 to 55.4)
1985–1989	173	33.0 (31.4 to 34.7)	183	48.2 (45.9 to 50.5)
1990–1994	391	31.7 (30.6 to 32.8)	299	49.6 (47.8 to 51.4)
1995–2000	649	32.2 (31.3 to 33.0)	226	49.4 (47.3 to 51.4)
		$P = .41$		$P = .21$

* Least squares mean age is given in years and is adjusted to the observed marginal distribution of the covariates. *P* values are for the F test using the type III sums of squares. CIS/AIS = carcinoma in situ or adenocarcinoma in situ; HPV = human papillomavirus; CI = confidence interval; — = not applicable.

have failed to obtain a Papanicolaou test within intervals recommended by current US screening guidelines. Thus, a majority of cancer cases caused by carcinogenic HPV types other than HPV16 or 18 will potentially be diagnosed if vaccinated women fail to attend regular screening.

In this study, we found evidence that the fraction of HPV16-positive cancers in New Mexico decreased from the early 1980s to the late 1990s. One possible explanation for this secular trend is that implementation of cytology-based screening and treatment led to this pattern. Cytology screening programs were initiated in the 1950s in the United States and achieved high coverage during the time period of this study (ie, 1980–2000). Rates of invasive cervical cancer in New Mexico peaked in the late 1970s, presumably when cytology screening was achieving broad population coverage, and has declined steadily from the 1980s onward (22). It is plausible that the most carcinogenic HPV genotype, HPV16, would be more likely to be detected and therefore censored for cancer outcomes by programs with good screening coverage. This may be especially true of HPV16 infection, which is more likely to result in abnormalities that trigger clinical action, such as HSIL cytology (21) and noticeable lesions on colposcopic examination (38). Ongoing surveillance to assess potential HPV16 and 18 disease replacement with nonvaccine HPV genotypes following widespread implementation of HPV16/18 vaccination must compare population-based HPV genotype–specific prevalence in women diagnosed with CIN1 or worse vs women with asymptomatic HPV infections (ie, normal cytology). Our results suggest that this surveillance must also adjust for the impact of changing screening practices on HPV genotype–specific time trends.

We confirmed and extended previous observations (4,39) that HPV18 is underrepresented in precancerous lesions compared with its presence in invasive cancers. Upon further examination, we found a more complex relationship between HPV phylogenetic species and different histologic types. Specifically, we found a general characteristic phenotype for alpha-7 HPV genotypes, which is composed of HPV18, 39, 45, 59, 68, and 70, because exclusion of HPV18 did not meaningfully change the prevalence ratio of invasive cancer to CIS/AIS. We also found that this high prevalence ratio was only observed in squamous cell histology, not in glandular cell histology.

The deficit of alpha-7 genotype–positive CIS has several possible explanations. Alpha-7 genotype–positive CIS appear to be harder to detect by cervical screening than CIS related to other HPV genotypes. Alternatively, as others have suggested, alpha-7 genotypes, especially HPV18, progress more rapidly from infection to invasive cervical cancer (4,40). There is also evidence suggesting that alpha-7 HPV genotypes are less likely to cause cytologic HSIL than other HPV genotypes. In particular, Khan et al. (41) noted a lag time in the development HPV18-related

Table 6. Predicted marginal proportions of HPV type from multinomial logistic regression models*

		Model 1†			Model 2‡		
		HPV16	HPV18	Other HPV genotypes	HPV16-related alpha-9	HPV18-related alpha-7	Other HPV species
Variable	N	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)
Year of diagnosis							
1980–1984	100	68.5 (59.5 to 77.5)	8.1 (3.4 to 12.8)	20.3 (12.1 to 28.5)	82.8 (76.1 to 89.5)	8.1 (3.8 to 12.4)	6.1 (1.2 to 11.0)
1985–1989	356	60.9 (55.8 to 66.0)	6.8 (4.3 to 9.3)	26.6 (21.9 to 31.3)	78.3 (74.2 to 82.4)	12.0 (8.7 to 15.3)	3.9 (1.9 to 5.9)
1990–1994	690	53.1 (49.4 to 56.8)	9.5 (7.3 to 11.7)	31.2 (27.7 to 34.7)	74.8 (71.7 to 77.9)	14.2 (11.7 to 16.7)	4.7 (3.1 to 6.3)
1995–2000	875	53.0 (49.7 to 56.3)	7.1 (5.3 to 8.9)	35.0 (31.9 to 38.1)	77.1 (74.2 to 80.0)	12.5 (10.1 to 14.9)	5.5 (3.9 to 7.1)
			$P = .009$			$P = .25$	
Age at diagnosis, y							
<20	67	60.4 (48.6 to 72.2)	5.5 (0.0 to 12.8)	29.3 (19.3 to 39.3)	74.5 (63.1 to 85.9)	13.9 (3.1 to 24.7)	7.0 (1.5 to 12.5)
20–29	593	56.7 (52.4 to 61.0)	8.8 (6.1 to 11.5)	29.6 (25.9 to 33.3)	78.7 (75.0 to 82.4)	12.5 (9.2 to 15.8)	3.8 (2.2 to 5.4)
30–39	604	58.6 (54.7 to 62.5)	9.1 (6.7 to 11.5)	29.7 (26.2 to 33.2)	79.6 (76.5 to 82.7)	14.3 (11.4 to 17.2)	3.6 (2.2 to 5.0)
40–49	306	53.9 (48.2 to 59.6)	11.7 (8.4 to 15.0)	27.9 (22.8 to 33.0)	72.2 (67.3 to 77.1)	17.0 (13.1 to 20.9)	4.3 (1.9 to 6.7)
50–59	178	50.4 (42.8 to 58.0)	6.9 (4.0 to 9.8)	37.0 (29.6 to 44.4)	71.5 (64.8 to 78.2)	14.2 (9.7 to 18.7)	8.4 (3.5 to 13.3)
60–69	146	45.1 (36.7 to 53.5)	3.6 (1.2 to 6.0)	43.5 (34.9 to 52.1)	75.0 (67.6 to 82.4)	8.4 (4.7 to 12.1)	8.6 (2.7 to 14.5)
70–79	86	44.5 (32.9 to 56.1)	4.9 (1.2 to 8.6)	43.8 (32.2 to 55.4)	74.9 (64.7 to 85.1)	6.6 (2.7 to 10.5)	12.0 (3.0 to 21.0)
≥80	41	40.4 (24.5 to 56.3)	1.3 (0.0 to 3.8)	46.0 (30.1 to 61.9)	60.8 (43.7 to 77.9)	1.2 (0.0 to 3.6)	26.7 (9.8 to 43.6)
			$P < .001$			$P < .001$	
Ethnicity							
Non-Hispanic white	1185	57.8 (55.1 to 60.5)	8.2 (6.6 to 9.8)	28.9 (26.4 to 31.4)	77.1 (74.7 to 79.5)	12.6 (10.8 to 14.4)	5.3 (3.9 to 6.7)
Hispanic	836	51.3 (48.0 to 54.6)	7.6 (5.8 to 9.4)	35.3 (32.2 to 38.4)	76.7 (74.0 to 79.4)	12.9 (10.5 to 15.3)	4.6 (3.2 to 6.0)
			$P = .01$			$P = .77$	
Histology							
Squamous cell	1823	57.1 (54.7 to 59.5)	6.2 (5.0 to 7.4)	33.0 (30.8 to 35.2)	79.8 (77.8 to 81.8)	11.3 (9.7 to 12.9)	5.2 (4.2 to 6.2)
Adenocarcinoma or adenosquamous	147	45.2 (36.6 to 53.8)	26.7 (18.9 to 34.5)	10.3 (4.8 to 15.8)	53.9 (45.5 to 62.3)	26.4 (19.0 to 33.8)	1.6 (0.0 to 4.0)
Other	51	29.5 (17.0 to 42.0)	10.8 (3.0 to 18.6)	42.3 (28.8 to 55.8)	56.7 (44.2 to 69.2)	16.6 (7.4 to 25.8)	9.5 (0.5 to 18.5)
			$P < .001$			$P < .001$	
Stage at diagnosis							
In situ	1213	55.0 (51.9 to 58.1)	5.3 (3.9 to 6.7)	35.7 (32.8 to 38.6)	82.2 (79.7 to 84.7)	7.2 (5.6 to 8.8)	6.7 (4.7 to 8.7)
Localized	477	58.6 (53.9 to 63.3)	10.5 (7.6 to 13.4)	25.0 (20.7 to 29.3)	71.8 (67.5 to 76.1)	18.5 (14.8 to 22.2)	3.9 (1.9 to 5.9)
Regional	269	54.0 (47.3 to 60.7)	14.5 (9.6 to 19.4)	24.7 (19.2 to 30.2)	63.7 (57.0 to 70.4)	26.6 (20.1 to 33.1)	2.9 (1.1 to 4.7)
Distant	47	44.0 (28.9 to 59.1)	13.5 (3.1 to 23.9)	30.1 (16.4 to 43.8)	60.4 (45.5 to 75.3)	27.5 (13.6 to 41.4)	0.0 (0.0 to 0.0)
Invasive, NOS	15	55.8 (30.9 to 80.7)	6.4 (0.0 to 17.0)	32.3 (8.6 to 56.0)	81.6 (63.0 to 100.0)	13.3 (0.0 to 30.9)	0.0 (0.0 to 0.0)
			$P < .001$			$P < .001$	

* HPV = human papillomavirus; CI = confidence interval; NOS = not otherwise specified. *P* values are for the Wald F test of the association between the variable (eg, age) and the distribution of HPV genotype or group of phenotypes.

† Categories are defined in a hierarchical manner: HPV16 > HPV18 > other HPV > HPV negative. Marginal proportions are not shown for the HPV negative category but can be obtained by summing the other three proportions and subtracting from 100.

‡ Categories are defined in a hierarchical manner: Alpha-9 > alpha-7 > other HPV > HPV negative. Marginal proportions are not shown for the HPV negative category but can be obtained by summing the other three proportions and subtracting from 100.

CIN3 or worse compared with HPV16-related CIN3 or worse, which argues in favor of poorer detection and against more rapid progression. We found that invasive cancers related to alpha-7 genotypes were more likely to be diagnosed with regional or distant metastasis than alpha-9–related cancers, further evidence of poorer detection of alpha-7–related disease. If poorer detection of alpha-7 precancerous lesions by cytology is the cause of the dearth of alpha-7–related CIS, we would anticipate that the use of HPV testing in primary cervical cancer screening could result in an increase in detection, assuming equal analytic sensitivity for alpha-7 and non–alpha-7 carcinogenic HPV genotypes.

Despite the overall decline in cervical cancer rates in Western countries, the decrease has been restricted to the more common squamous cell carcinoma and there is evidence of increasing rates of adenocarcinoma in the United States (42) and Europe (43). The etiology of these increases remains unexplained, although several hypotheses are plausible based on epidemiological risk factors (44–46). In this study, as previously reported (2–7), we found that alpha-7 HPV genotypes were more strongly associated with adenocarcinoma than alpha-9 HPV genotypes. These data, along with evidence that other factors (44–46), including contraceptive hormones, may elevate the risk for adenocarcinoma relative to that for squamous cell carcinoma and the secular increases in exposure to these risk factors may explain the increase in adenocarcinoma in Western countries. Studies of the molecular and statistical interactions between the use of oral contraceptive hormones and alpha-7 HPV genotypes may help elucidate the underlying biological mechanisms.

Our study has some limitations. First, our study was restricted to Hispanic and non-Hispanic white women residing in New Mexico, and thus our findings may not be generalizable to other populations. Second, because of the cross-sectional nature of this study, we can only infer relationships between time and

outcomes. For example, our data suggest that cases positive for HPV genotypes other than HPV16 and 18 arise on average at an older age than those positive for HPV16 and 18. There is clearly a wide variation in the sojourn time between the causal infection and the diagnosis of cancer across the carcinogenic HPV genotypes, which may preclude using the knowledge of HPV genotypes to reliably predict the age-specific risks of invasive cancer. It is noteworthy that our finding of a difference in the mean ages at diagnosis between cancers positive for HPV16 or 18 and those positive for other HPV genotypes applies only to (relatively) well-screened populations such as this population study conducted in New Mexico. Low rates of progression from CIS/AIS to cancer and the age at diagnosis are undoubtedly strongly influenced by the censoring of CIS/AIS diagnoses, which is probably confounded by the carcinogenicity of each HPV genotype and the ability to detect HPV-related CIS/AIS in a timely fashion. To examine the impact of screening on HPV genotype distributions in cervical cancer, ongoing large international case series should analyze the distribution of HPV genotypes in relation to the history and quality of screening. Third, interpretation of the case–control odds ratio as a relative risk requires that the relative prevalence of HPV genotypes in the control sample be equal to the relative exposure to HPV genotypes in the source population of the cases. Our control subjects were selected over a time period (1996–2000) that was more recent than the time period during which most of the case subjects were presumably exposed to HPV. Thus, the interpretation of our odds ratios as relative risks requires that the relative prevalence of the circulating HPV genotypes remain stable over an extended time period. However, because we used HPV16-positive subjects as the referent group, it is not necessary to assume that the absolute prevalence of HPV has remained static. Recent data from A Randomized Trial In Screening to Improve Cytology (ARTISTIC) (47) lend support to the assumption of stability in the relative prevalence of HPV genotypes across time even if the overall prevalence of HPV infections has increased during the past 20–40 years as has been suggested by some (48). In the ARTISTIC trial, there were relatively minor differences in HPV genotype distribution between HPV-positive women 40 years or older and those younger than 40 years. Fourth, the prevalence of HPV genotypes in the control sample is a point-in-time prevalence and may differ from the cumulative exposure of the case subjects to these HPV genotypes. For the less frequently observed HPV genotypes, the control sample may underestimate the cumulative exposure in the source population of the cases and result in an overestimate of the relative risk. Finally, we also note that multiple HPV genotypes were detected in 16% of the samples. These multiple coinfections undoubtedly led to misattribution of some cases of CIS/AIS and cancer to HPV16 and 18, which would be expected to inflate the relative risks and diminish the differences in ages at diagnosis for HPV16- and HPV18-related cancers. A recent systematic review of the published HPV genotype prevalence data among cervical, vaginal, and vulvar precancers and cancers in the United States highlighted issues related to HPV coinfections when assigning disease attribution (49).

This study of HPV genotypes in New Mexico provides important baseline data for evaluating the effectiveness of newly implemented HPV-based technologies, HPV vaccines, and HPV screening in the prevention of cervical cancer. Moreover, these data can guide the future application of these technologies to maximize the cost-effective, public health benefits of these interventions.

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Notes

C. M. Wheeler, the study Principal Investigator, had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. W. C. Hunt performed the biostatistical analyses for the study. N. E. Joste, the study pathologist, reviewed all study slides for initial case selection and for blinded review of tissue sections flanking sections obtained for laboratory HPV studies. C. R. Key supported the development of the Tissue Acquisition Section (TAS), which identified and ascertained all case materials. W. G. V. Quint conducted blinded SPF-based laboratory analyses. P. E. Castle prepared the initial manuscript draft and participated in critical study analyses. All authors provided critical input to the analyses and manuscript drafting process and approved the final manuscript submitted.

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