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Associations between human papillomavirus and history of cancer among U.S. adults in the National Health and Nutrition Examination Survey (2003–2010)

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Background: Human papillomavirus (HPV) is an infectious agent that has been associated with human cancer. We have updated the U.S. population sero-prevalence using a large National Health and Nutrition Examination Survey (NHANES) sample of adults from 2003 to 2010, and have analysed the associations between HPV seropositivity and self-reported history of cancer.

Methods: Four cross-sectional cycles (2003–2004, 2005–2006, 2007–2008, and 2009–2010) were used, for a total of 12 759 participants who had both cancer history and HPV serum information.

Results: The sero-prevalences of HPV types 6, 11, 16, and 18 were 15.0%, 4.8%, 11.5%, and 4.1%, respectively. Females had significantly higher HPV prevalence than males ($P<0.05$) for all subtypes. Positive associations between HPV 16/18 seropositivity and lifetime history of any cancer (adjusted odds ratio-$OR_{adj}=1.68$; 95% CI: 1.35, 2.01), history of any of eight selected cancers ($OR_{adj}=2.63$; 95% CI: 1.78, 3.90), lung cancer ($OR_{adj}=5.14$; 95% CI: 1.29, 20.44), and cervical cancer ($OR_{adj}=2.55$; 95% CI: 1.63, 3.98) were observed.

Conclusions: The finding of significant associations between HPV 16/18 seropositivity and lifetime history of cancer adds epidemiological evidence to the carcinogenicity potential of HPV 16 and 18 in other tissues. With increasing coverage of the HPV vaccine in the U.S., future NHANES data and sample collection may allow further detailed evaluation of the population impact of the HPV vaccination on cancer prevention.

Human papillomavirus (HPV) is an infectious agent that has been associated with human cancer (zur Hausen, 1996). There are more than 100 HPV subtypes, some of which, like HPV 16 or HPV 18, were identified as oncogenic or high-risk types after the epidemiologic observation that not all the cervical lesions evolve into cervical cancer (zur Hausen, 1986, 2002). HPV 16 and 18 have been associated with cervical and anogenital cancers (zur Hausen, 1996), and in more recent years to other cancer types, such as lung (Syrjanen, 2012; Ragin *et al*, 2014), head and neck (Gillison *et al*, 2000), and prostate cancers (Dillner *et al*, 1998; Whitaker *et al*, 2013).

Overall HPV sero-prevalence in the United States has been described using the 2003–2004 National Health and Nutrition Examination Survey (NHANES) data (Stone *et al*, 2002; Markowitz *et al*, 2009), or has been ascertained using cervical swabs among women, in an attempt to assess the effect of HPV vaccination (Markowitz *et al*, 2013); the population sero-prevalence of HPV 16 alone has also been published (Stone *et al*, 2002). We are updating the population sero-prevalence using a large NHANES sample of adults from 2003 to 2010.

Given that sero-prevalence of HPV 16 or 18 provides reasonable estimates of cumulative exposure (Chua *et al*, 1996; af

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Geijersstam *et al*, 1998; Markowitz *et al*, 2009, 2013), and the implication of HPV as an aetiologic risk factor for some major human cancers, we are also analysing the associations between HPV seropositivity and self-reported history of cancer. We hypothesised that seropositivity of HPV 16 or 18 is associated with a higher frequency of personal history of cancer compared with seronegativity.

MATERIALS AND METHODS

Study population and design. The NHANES surveys were conducted by the U.S. National Center for Health Statistics (NCHS) of the Centers for Disease Control and Prevention (CDC) to assess the health and nutritional status of adults and children in the U.S. The NHANES study protocol has been described previously in detail by by CDC (Zipf *et al*, 2013). In brief, civilians, non-institutionalized persons in the U.S. aged 2 months or older were eligible to participate. They were selected through a stratified, multi-stage, probability-cluster design. The survey's components were administered in homes and in mobile examination centres, and also provided national reference data for biological markers, anthropometric measures, as well as demographic and socio-economic status data.

Four cross-sectional cycles (2003–2004, 2005–2006, 2007–2008, and 2009–2010) were used and combined using NCHS recommendations (NCHS, 2008, 2013). Of the initial 41 156 participants, those who lacked information on either only HPV serum ($n=9223$), only on history of cancer ($n=1562$), or both ($n=17\,455$) were excluded. Among the remaining 12 916 participants who had both cancer and HPV serum information, 45 and 112 participants from the 2007–2008 and 2009–2010 survey cycles, respectively, received HPV vaccination, and these 157 people were also excluded. The final population consisted of 12 759 adults aged 18–59 years. The NHANES protocol was developed and reviewed in compliance with the policies for protection of human research subjects developed by the U.S. Department of Health and Human Services (Zipf *et al*, 2013). The Institutional Review Board of the Feinstein Institute for Medical Research determined that this study is IRB exempt.

History of cancer. Lifetime cancer history was defined based on a positive answer to the question in the medical condition questionnaire: 'Have you ever been told by a doctor or other health professional that you had cancer or a malignancy of any kind?'.

Eight specific cancers that were potentially linked to HPV were identified based on positive answers to the subsequent questions 'What kind of cancer?'; they were cervix, oesophagus, larynx/windpipe, lung, mouth/tongue/lip, ovary, prostate, and rectum. No information on vulvar, anal, penile, or vaginal cancers was collected. An additional dichotomised cancer variable (eight selected cancer) was created which included a positive answer to having had any of those eight cancers.

Serum HPV. All NHANES participants aged 18–59 years were eligible for serologic testing. Serum samples were analysed using the Luminex platform to simultaneously assay antibodies to HPV 6, 11, 16, and 18, and were detected by a multiplexed, competitive immunoassay by displacement of fluorescently tagged neutralising monoclonal antibody from virus-like particles (VLP)-coated microspheres (Opalka *et al*, 2003; Dias *et al*, 2005). Dichotomized serostatus (positive or negative) were defined by NHANES based on cutoff values (mMU ml^{-1}): $\geqslant 20$, $\geqslant 16$, $\geqslant 20$, and $\geqslant 24$ for types 6, 11, 16, and 18, respectively. For analytic purposes, three additional HPV variables were created: positivity to any of the four HPV types (HPV any four), HPV 6/11, and HPV 16/18, with the later two representing low- and high-risk HPV types, respectively.

Other covariates. The associations between cancer and HPV serostatus were adjusted for the following *a priori* possible confounders: sex, age, race, education, poverty income ratio (PIR), body mass index (BMI), smoking status, and alcohol use. Age was categorised into four groups: 18–29, 30–39, 40–49, and 50–59 years. Race included three categories: non-Hispanic white, non-Hispanic black, and other, where other consisted of mainly Mexican-American and other Hispanic, as well as other races (including multi-racial subjects). The latter category represented approximately 3–6% of the total sample in each survey cycle. Education was grouped into three categories: greater, equal, or less than high school level, where high school was defined as having 12 years of primary and secondary school. PIR in NHANES was calculated by dividing family income by the poverty level issued by the Department of Health and Human Services, which is specific to family size, the appropriate year and state. In this study, PIR was grouped into three categories: <1, $1\leqslant \text{PIR}\leqslant \text{median}$, $>\text{median}$, where medians ($\sim$2.3–2.7) were calculated based on the PIR$\geqslant 1$ for each survey cycle. BMI was grouped into two categories based on the cutoff value of 25 kg m^{-2}, where BMI $\geqslant$25 kg m^{-2} indicates overweight and obese weight status based on commonly used health guidelines (CDC; NIH). Dichotomised smoking status was based on positive answers to the question, 'Have you smoked at least 100 cigarettes in your entire life?'. Dichotomised alcohol use was based on positive answers to the question, 'Have you had at least 12 alcohol drinks per 1 year?'.

Statistical methods. The sero-prevalences of HPV types were calculated for the overall data, as well as stratified by sex, race, age groups, survey cycles, and cancer status. The HPV–cancer associations were tested using logistical regression, and models were fit with one cancer variable at a time. The crude model was fit with each of the seven HPV indicators as the only predictor (one at a time), to obtain a preliminary understanding of the HPV–cancer associations. The adjusted models were run with HPV 16/18 only, as 16 and 18 are high-risk viral subtypes. Models run on the whole-data set consisted of both males and females, except for cervical cancer and ovarian cancer, which were run on a female-only data set, and prostate cancer, which was run on a male-only data set.

Statistical analyses were performed using SAS software (version 9.3, Cary, NC, USA) and sampling weights were applied to account for the complex sampling NHANES design according to NHANES guidelines (NCHS, 2013).

RESULTS

Table 1 lists the weighted characteristics of the participants 18–59 years of age from NHANES 2003–2010 included in this study ($n=12\,759$). The weighted mean age ($\pm$s.e.) of the participants was 39.6 $\pm$ 0.16 years. Half of the participants were females and about 66% of the total population was overweight or obese (BMI$\geqslant$25 kg m^{-2}). Non-Hispanic whites accounted for 68% of the total population; 11.5% were non-Hispanic blacks. Approximately 19% of the participants were from families with income below the poverty level (PIR<1), and 17% of the participants had lower than high school education. Non-smokers and non-drinkers accounted for 52% and 22% of the total population, respectively. The prevalence ($\pm$s.e.) of lifetime history of cancer, any of the eight selected cancers, and cervical cancer were 4.88 $\pm$ 0.26%, 1.44 $\pm$ 0.16%, and 0.97 $\pm$ 0.12%, respectively. The prevalence of the other cancer types was below 0.13%.

The sero-prevalences of HPV types 6, 11, 16, and 18 were 15.0%, 4.8%, 11.5%, and 4.1%, respectively, and remained relatively stable across the four survey cycles between 2003 and 2010: prevalence ranges were 12.8–17.2%, 4.4–5.4%, 11.1–11.8%, and 3.7–4.6% for HPV types 6, 11, 16, and 18, respectively. Females had significantly

Table 1. Weighted characteristics of the study population—National Health and Nutrition Examination Survey (NHANES) 2003–2010

Characteristic	Number	Weighted percent	s.e.
Sex			
Males	6180	49.98	0.39
Females	6579	50.02	0.39
Age			
18–29 years	3341	23.72	0.58
30–39 years	3264	24.66	0.56
40–49 years	3331	27.66	0.55
50–59 years	2823	23.96	0.64
Race			
White	5853	67.99	1.77
Black	2602	11.52	0.88
Others	4304	20.49	1.39
Education			
<high school	3231	16.71	0.66
=high school	3041	24.03	0.61
>high school	6478	59.26	0.94
Poverty income ratio			
<1	3444	18.68	0.67
1⩽PIR⩽median	4060	26.46	0.71
>median	5255	54.86	0.97
Body mass index			
$\geqslant 25\,kg\,m^{-2}$	8743	65.95	0.69
Smoking			
Yes	5904	48.11	0.86
Alcohol use			
Yes	8690	78.01	0.84
Lifetime cancer			
Yes	508	4.88	0.26
Selected eight cancers			
Yes	177	1.44	0.16
Cervical cancer			
Yes	124	0.97	0.12
Oesophageal cancer			
Yes	2	0.02	0.02
Larynx/windpipe cancer			
Yes	2	0.04	0.03
Lung cancer			
Yes	11	0.09	0.03
Mouth/tongue/lip cancer			
Yes	5	0.05	0.02
Ovarian cancer			
Yes	20	0.13	0.04
Prostate cancer			
Yes	14	0.13	0.05
Rectal cancer			
Yes	1	0.01	0.01

higher HPV prevalence than males ($P<0.05$) for all subtypes (Figure 1). The sero-prevalence of HPV 6/11 was 17.2% overall, and was 24.9% and 9.5% among females and males, respectively. The sero-prevalence of HPV 16/18 was 14.0% overall, and was 21.4% and 6.7% among females and males, respectively. The sero-prevalence of any of the four HPV types was 25.5% overall, and was 36.6% and 14.4% among females and males, respectively. The sero-prevalences of the HPV types also varied by age (Figure 2) and by race/ethnicity (Figure 3), with elevated sero-prevalences seen among those aged 30–39 years and among blacks.

Compared with non-cancer cases, sero-prevalences of HPV 16/18 were generally higher among participants with a lifetime history of cancer (Table 2), for history of any of the eight selected cancers, cervical, lung, and ovarian cancer. In general, the prevalence of HPV 16/18 among participants with a history of cancer was 1.6–3 times higher than the overall HPV 16/18 prevalence (14%). Uncertainties of the estimates for history of other cancers are large, likely because of the limited number of cancer cases ($n<5$) and/or large standard errors (>30%).

Results from the crude logistic models showed significant positive associations between HPV 16/18 serum positivity and the following variables: lifetime history of cancer, history of any of the eight selected cancers, cervical, and lung cancers. HPV 16/18 serum positivity was not significantly associated with larynx/windpipe, mouth/tongue/lip, ovarian, and prostate cancers (data not shown). Due to the small sample size of oesophageal (two cases) and rectal cancer (one case) cases, no reliable results were found as the convergence criteria required to run the model were not met.

Multivariable analysis. For the study population as a whole, positive associations between HPV 16/18 seropositivity and lifetime history of any cancer (adjusted odds ratio-$OR_{adj}=1.68$; 95% CI: 1.35, 2.01), history of any of the eight selected cancers ($OR_{adj}=2.63$; 95% CI: 1.78, 3.90), and lung cancer ($OR_{adj}=5.14$; 95% CI: 1.29, 20.44) were observed (Table 3). Age and sex were two common significant covariates in the adjusted models for these three types of cancer. Among females, similar positive associations were found between HPV 16/18 seropositivity and history of cancer ($OR_{adj}=1.57$; 95% CI: 1.22, 2.01), history of any of the eight selected cancers ($OR_{adj}=2.57$; 95% CI: 1.64, 4.03), and lung cancer ($OR_{adj}=5.04$; 95% CI: 1.34, 18.92). In addition, HPV 16/18 was found to be a significant predictor for cervical cancer ($OR_{adj}=2.55$; 95% CI: 1.63, 3.98). Among males, HPV 16/18 positivity was borderline significantly associated only with lifetime history of cancer ($OR_{adj}=1.81$; 95% CI: 0.98, 3.33).

DISCUSSION

We examined the associations between HPV serum infection and history of cancer among a large population sample of U.S. adults aged 18–59 years. HPV infection with two of the high-risk strains (HPV 16 or HPV 18) was associated with lifetime history of cancer, history of eight selected cancers, and lung cancer in the study population as a whole and among females, as well as with cervical

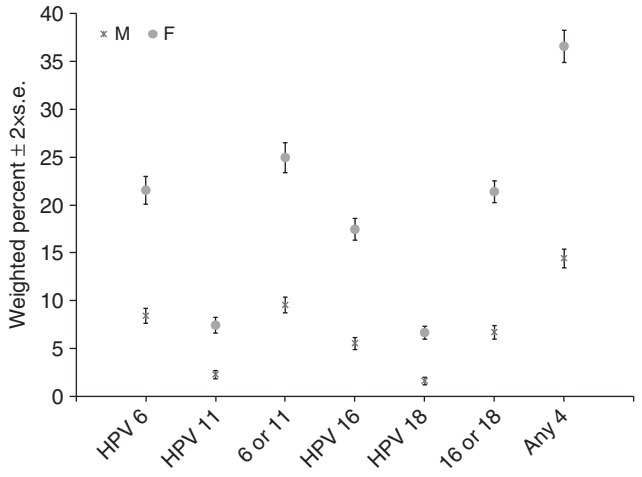

Figure 1. Weighted sero-prevalences (±2 × s.e.) of human papillomavirus (HPV) types 6, 11, 16, and 18 according to sex—NHANES 2003–2010.

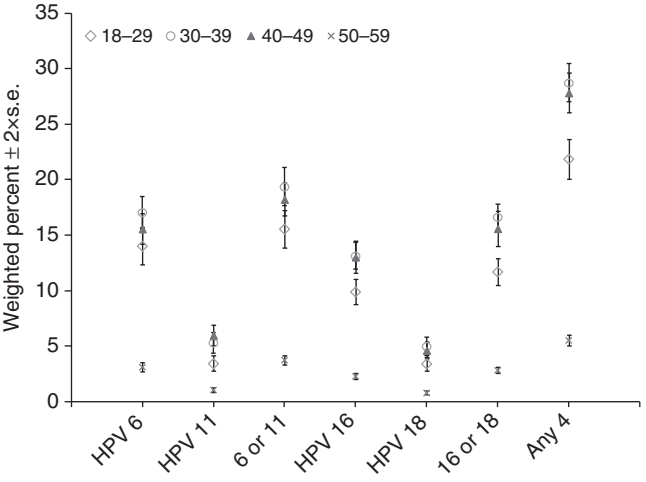

Figure 2. Weighted sero-prevalences (±2 × s.e.) of human papillomavirus (HPV) types 6, 11, 16, and 18 according to age (years)—NHANES 2003–2010.

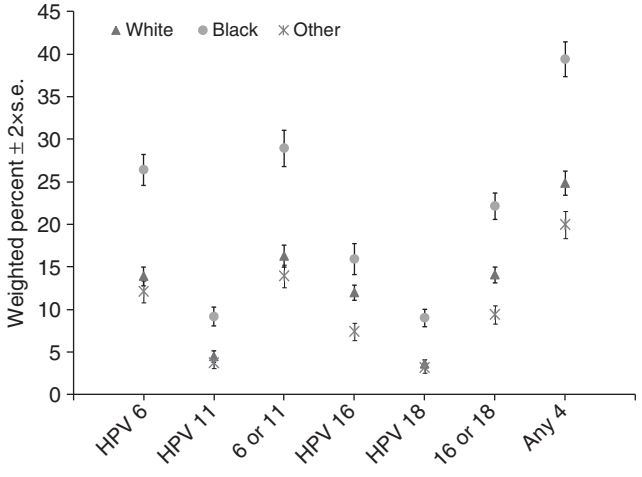

Figure 3. Weighted sero-prevalences (±2 × s.e.) of human papillomavirus (HPV) types 6, 11, 16, and 18 according to race/ethnicity—NHANES 2003–2010.

Table 2. Prevalence of seropositivity of human papillomavirus (HPV) types 16 or 18 according to cancer history (National Health and Nutrition Examination Survey (NHANES) 2003–2010)

Variable	HPV 16/18	Number	Weighted percent HPV 16/18 +	s.e.
Lifetime history				
Cancer	+/–	130/378	22.8	1.8
Non-cancer	+/–	1757/10 494	13.6	0.3
Eight selected				
Cancer	+/–	66/111	37.6	4.1
Non-cancer	+/=	1821/10 761	13.7	0.3
Cervix				
Cancer	+/-	52/72	42.5	5.1
Non-cancer	+/-	1835/10 800	13.7	0.3
Oesophagus				
Cancer	+/–	0/2		
Non-cancer	+/–	1887/10 870	14.0	0.3
Larynx				
Cancer	+/–	1/1	49.9	35.4
Non-cancer	+/–	1886/10 871	14.0	0.3
Lung				
Cancer	+/–	5/6	42.4	18.4
Non-cancer	+/–	1882/10 866	14.0	0.3
Mouth				
Cancer	+/–	1/4	30.6	24.5
Non-cancer	+/–	1886/10 868	14.0	0.3
Ovary				
Cancer	+/–	6/14	25.5	9.2
Non-cancer	+/–	1881/10 858	14.0	0.3
Prostate				
Cancer	+/–	1/13	10.3	10.0
Non-cancer	+	1886/10 859	14.0	0.3
Rectum				
Cancer	+/–	1/0	100.0	0.0
Non-cancer	+/–	1886/10 872	14.0	0.3

cancer among females. These associations remained significant after adjusting for some possible confounders, such as age, race, smoking, and drinking status.

The finding of significant positive associations between HPV 16/18 seropositivity and cervical cancer is not surprising, given the well-established aetiologic role that HPV 16 and 18 play in the development of cervical cancer (Clifford *et al*, 2003; Bosch *et al*, 2008). National surveillance data have shown higher incidence of cervical cancer among blacks (Watson *et al*, 2008); similarly, in the present analysis, non-Hispanic blacks had the highest HPV 16/18 seropositivity. A similar trend has also been reported in a previous analysis of NHANES (2003–2004) participants aged 14–59 (Markowitz *et al*, 2009). However, our data suggest that race was not a significant predictor of the association between HPV seropositivity and history of cervical cancer in the multivariable model.

Table 3. Associations between seropositivity of human papillomavirus (HPV) types 16 or 18 and selected history of cancer outcomes

Cancer	Adjusted odds ratio[a]	95% confidence interval		Sex[b]
Lifetime	1.68	1.35	2.09	F + M
	1.81	0.98	3.33	M
	1.57	1.22	2.01	F
Eight selected	2.63	1.78	3.90	F + M
	2.57	0.67	9.83	M
	2.57	1.64	4.03	F
Cervix	2.55	1.63	3.98	F
Lung	5.14	1.29	20.44	F + M
	5.04	1.34	18.92	F

[a]Models were adjusted for sex, age, education, race, poverty income ratio (PIR), body mass index (BMI), smoking status, and alcohol-use status. Sampling weights were applied to account for the complex sampling National Health and Nutrition Examination Survey (NHANES) design.
[b]Models were run on the whole population with both females and males (F + M), and for male-(M) or female-(F) only data sets.

The fact that the association between history of cervical cancer and HPV seropostivity is weak could be explained in part by cervical cancer aggressiveness, patterns of cervix cancer screening and follow-up/treatment services (Watson *et al*, 2008), as well as heterogeneity and misclassification in cervical cancer, since pre-cancerous cervical lesions discovered through routine screenings are often perceived by women as 'cancer'.

The link between HPV infection and lung cancer is still debated. The present analysis shows a significant association between a history of lung cancer and HPV 16/18 serological positivity in females. This association is in contrast with results from a large longitudinal nested case-control study of Finnish women (Simen-Kapeu *et al*, 2010), which reported no increased risk of lung cancer with serum HPV 16/18 infection. The discrepancies in study design and the method used for the estimate of smoking (self-reported *vs* cotinine level) may have contributed to the differences observed between the present study and the Finnish study. A recent pooled analysis suggested that viral integration in the DNA, a sign of carcinogenicity, was almost exclusively present among females compared with males, but in a small sample of lung cancer cases (Ragin *et al*, 2014). Other studies using HPV DNA have suggested a high prevalence of HPV in lung cancer tumours compared with normal tissues (Klein *et al*, 2009; Srinivasan *et al*, 2009; Syrjanen, 2012; Ragin *et al*, 2014). *In vitro* studies also showed that benzo[a]pyrene, a major carcinogen in cigarette smoke, enhances virion syntheses of HPV 16 and 18 (Alam *et al*, 2008). However, a recent study (Anantharaman *et al*, 2014) found no association between HPV antibodies or HPV DNA and lung cancer.

The significant associations between HPV 16/18 seropositivity and a comprehensive variable including eight selected cancer types (cervix, oesophagus, larynx/windpipe, lung, mouth/tongue/lip, ovary, prostate, and rectum) supports the notion that those subjects with HPV 16/18 seropositivity may be at higher risk for these cancers. While estimates for each of these specific cancer types could not be calculated in this data set due to small sample sizes of captured cancer cases, our result is consistent with those from cancer type-specific studies. For instance, research based on HPV DNA data has also shown increases in the population level incidence of HPV-positive oropharyngeal cancers in the U.S. (Chaturvedi *et al*, 2011), Sweden (Nasman *et al*, 2009), and Canada (Nichols *et al*, 2013). A wide range of elevated HPV prevalence has also been reported, albeit with large variations, for cancer tissues such as ovarian (15.5%, ranged = 0–66.7%; (Svahn *et al*, 2014)), oesophageal (30.9%; (Syrjanen & Syrjanen, 2013)), as well as prostate cancers (HPV 16/18 sero-prevalence: 0.04–10.3%; (Dillner *et al*, 1998)).

The finding of significant associations between HPV 16/18 seropositivity and lifetime history of cancer adds epidemiological evidence to the carcinogenicity potential of HPV 16 and 18 on other tissues in addition to anogenital and oropharyngeal sites. The observed HPV–cancer association was also consistent with the high HPV 16/18 sero-prevalence seen in this study among participants with a history of cancer in comparison with those without any cancer.

The results of this study represent one of the most comprehensive pictures of U.S. population-based HPV infection in the context of cancer. The data consisted of four NHANES surveys between 2003 and 2010. In addition, both males and females were included in the analysis. A major limitation is the cross-sectional nature of the survey, which prevented the examination of temporal associations between HPV infection and cancers, and the fact that only antibodies to L1, the HPV structural proteins, were assessed in NHANES. It was not possible to determine the time frame between HPV testing and cancer occurrence. It was also not clear whether the observed seropositivity represented recent, latent, or reactivation of infection, and to what extent the natural history of seroreactivity differed between controls and cancer cases, whose immune response may be compromised due to cancer treatment. While HPV capsid antibody levels are stable during several years of follow-up (af Geijersstam *et al*, 1998) and HPV 16/18 had relatively persistent antibody responses, although with some variations (Carter *et al*, 2000; Ho *et al*, 2004), the use of HPV seropositivity in aetiological cancer studies is still emerging and need to be confirmed in tumour tissues. Other limitations include the rudimentary assessment of smoking history which is the main risk factor for many cancer sites, and the omission of other potential confounders such as lifetime number of sex partners, which has been shown to be associated with a increased risk of HPV infection (Markowitz *et al*, 2009) and is likely associated with smoking status as well. The relatively small number of cases for some cancers, and the fact that the outcomes were based on self-reported history, and for selected common cancers only, may have contributed to the wide error bars and confidence intervals observed. In addition, for some lethal cancer types, such as ovarian or lung cancer, a personal history of cancer likely captures only those who survived, thus introducing a survivorship bias. This bias, together with recall bias, may have affected the relative magnitude of the associations observed; for example, the association between HPV infection and history of lung cancer is unusually stronger than the association HPV–cervical cancer. It is possible that lung cancer survival differs according to the presence of HPV infection, as is well established for head and neck cancer, thus selecting for survival only those cases that are HPV positive.

However, our results suggest an association in the U.S. general population between seropositivity of high-risk HPV types (16/18) and lifetime history of cancer, cancer of the cervix and lung, and history of any of eight cancers that were selected because of their potential association with HPV. Further investigations into these associations using tumour tissues, a larger sample size, and a prospective design are warranted. With increasing coverage of HPV vaccine in the U.S., future NHANES data and sample collection may allow further detailed evaluation of the HPV–cancer relationship, as well as the population impact of HPV vaccination on cancer prevention.

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