

## Research Article

Smoking and Human Papillomavirus (HPV) Infection  
in the HPV in Men (HIM) Study

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## Abstract

**Background:** The influence of smoking on the natural history of HPV infection in men is not well understood. Smoking could influence the incidence and persistence of HPV infections by suppressing local immune function, increased cellular proliferation, upregulated proinflammatory factors, or induced host DNA damage resulting in increased susceptibility to infection. The purpose of this analysis is to assess prevalent HPV infections by smoking status in men, and to determine baseline risk of HPV infection associated with smoking.

**Methods:** The HPV in Men (HIM) study is a multinational prospective study of the natural history of HPV infections in men. Samples from the coronal sulcus, glans penis, shaft, and scrotum were combined for HPV DNA testing. Multivariable logistic regression was used to assess the association between smoking and any-, oncogenic-, and nononcogenic HPV infections.

**Results:** Our analyses revealed that current smoking was associated with an increased risk of any HPV infection (OR = 1.19; 95% CI: 1.01–1.41) and oncogenic HPV infection (OR = 1.24; 95% CI: 1.05–1.47). However, the association between smoking and any HPV infection (OR = 1.35; 95% CI: 1.05–1.73) and oncogenic HPV infection (OR = 1.46; 95% CI: 1.11–1.92) was only evident among men reporting fewer lifetime sexual partners.

**Discussion:** These results suggest that current smokers with the fewest number of sexual partners are associated with an increased risk for oncogenic HPV infection.

**Impact:** The relationship between smoking and HPV infection remains understudied in men; these data shed new light on the interplay between smoking, sexual activity, and risk of HPV infection. *Cancer Epidemiol Biomarkers Prev*; 21(1); 102–110. ©2011 AACR.

## Introduction

Human papillomavirus (HPV) is one of the most common sexually transmitted infections, with more than 6 million new infections occurring annually in the United States (1, 2). More than 120 different HPV types have been identified, more than 30 of which are transmitted through sexual contact. In addition to the diseases HPV causes directly in men, including genital warts and various cancers, the HPV virus is readily transmitted from men to women and can affect disease risk in women (3–6). However, most HPV infections are transient and asymptomatic or subclinical, do not result in disease, and are usually self-cleared.

Previous epidemiology studies have provided widely varied estimates of HPV prevalence in men ranging from

0% to 73% (7). Although prevalence findings are mixed, even less is known about the relationship between HPV and smoking in men. In studies conducted among women, smoking has been associated with longer duration of oncogenic HPV infections and increased risk of invasive cervical cancer (8–16). Thus, since approximately one-third of men worldwide are active smokers (17) and HPV seems to be highly prevalent in men, it is important to determine the potential influence of smoking on the natural history of HPV infection in men. Smoking could influence the incidence and persistence of HPV infections by suppressing local immune function, increased cellular proliferation and turnover, upregulated proinflammatory factors, or induced host DNA damage resulting in increased susceptibility to infection (18–34). The purpose of this analysis was to assess prevalent HPV infections by smoking status in men and determine the baseline risk of HPV infection associated with smoking.

## Materials and Methods

## Study population

The HPV in Men (HIM) study is a prospective study of the natural history of HPV infections in men in 3 countries. A full description of cohort methods and procedures has

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been previously published (35, 36). Briefly, men aged 18 to 70 years were recruited from Tampa, Florida, São Paulo, Brazil, and Cuernavaca, Mexico. Eligibility criteria included no previous diagnosis of penile or anal cancers, no previous diagnosis of genital or anal warts, had not participated in an HPV vaccine study, no previous diagnosis of HIV, no current penile discharge or burning during urination, not under current treatment for sexually transmitted infection, no history of imprisonment or homelessness during the past 6 months, no drug treatment during the past 6 months, no plans to relocate in the next 4 years, and willingness to attend 10 visits scheduled every 6 months for 4 years.

Men were recruited from 3 different population sources: the general population, universities, and organized healthcare systems. In Brazil, men were recruited from a large clinic in São Paulo that provides genitourinary healthcare and from the general population through television, radio, and newspaper advertisements. In Mexico, men were recruited in Morelos state, through a large government healthcare system, from factories, and the military. In the United States, men were mainly recruited from the University of South Florida and the general community in Tampa, FL. Human subjects' committees of the University of South Florida, Tampa, FL, the Ludwig Institute for Cancer Research, São Paulo, Brazil, the Centro de Referencia e Treinamento de Doenças Sexualmente Transmissíveis e AIDS, São Paulo, Brazil, the Instituto Mexicano del Seguro Social, and the National Institute of Public Health of Mexico, Cuernavaca, Mexico, approved all study procedures.

#### Data and sample collection

Eligible men provided written informed consent and underwent a clinical examination at a visit 2 weeks before the enrollment visit and every 6 months thereafter. Only men who returned for the enrollment visit were included in the study. At each visit, participants completed a computer-assisted self-interview questionnaire. Samples of penile and scrotal cells were obtained at each visit for detection of HPV DNA by use of PCR and subsequent genotyping. To encourage compliance with follow-up, men in the United States and Brazil were compensated for their participation.

#### Risk factor questionnaire

At enrollment an extensive sexual history and health questionnaire was administered which collected sociodemographic characteristics, sexual history, condom use practices, and alcohol and tobacco use. The questionnaire was self-administered using computer-assisted self-interviewing (CASI) and was typically completed in approximately 20 minutes. Never smokers were defined as men who had smoked less than 100 cigarettes in their lifetime. Former smokers were defined as men who had smoked at least 100 cigarettes in their lifetime but quit smoking at least 1 year before the baseline interview. Pack-years

smoked were calculated using the average number of cigarette packs smoked per day and the numbers of years smoked.

#### HPV penile and scrotal sampling

To maximize sampling and prevent fraying of applicators, 3 different prewetted Dacron applicators were used to sample the external genitalia and combined into a single sample for HPV detection. This method has been shown to maximize HPV detection and result in reproducible detection of genital HPV in men (37, 38). The study clinician at each site first swept 360° around the coronal sulcus and then another 360° around the glans penis and placed the swab into a collection vial containing Specimen Transport Medium (STM, Digene Corp.). A second swab was used to sample the entire skin surface of each of the quadrants of the shaft of the penis (left and right ventral, and left and right dorsal) and placed into a separate collection vial. A third swab was used for scrotum sampling. All 3 swabs were placed in a separate collection vial. Among men who were uncircumcised, the foreskin was sampled at the time of collection of the coronal sulcus/glans penis sample. All HPV samples were stored at -70°C until PCR analyses and genotyping were conducted. Prior to DNA extraction, the 3 samples were combined to produce one DNA extract per participant clinic visit.

#### DNA extraction and HPV genotyping

DNA extraction was accomplished using the QIAamp DNA Mini Kit (Qiagen) and HPV testing of the combined DNA sample was conducted using PCR for amplification of a fragment of the HPV *L1* gene. Briefly, 200 µL aliquots of clinical material were digested with 20 µL of proteinase K solution for 1 hour at 65°C, followed by 200 µL of lysis buffer. Specimens were tested for the presence of HPV by amplifying 50 µL of the DNA extracts using the Linear Array HPV genotyping test following manufacturer instructions (Roche Diagnostics). Samples were amplified using Perkin-Elmer GeneAmp PCR System 9700 as directed by the linear array protocol. HPV genotyping was conducted on all samples regardless of HPV PCR result. The 96.8% of specimens obtained were positive for β-Globin.

#### Statistical analysis

Three HPV categories (i.e., "Any HPV," "Oncogenic HPV," and "Only nononcogenic HPV") were used as the dependent variables in our analyses. A participant was considered positive for "any HPV" if he tested HPV-positive by PCR or tested positive for at least 1 genotype. The "Oncogenic HPV" category included men who were positive for at least one of the 13 oncogenic types tested for (HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 66) and included men infected with both oncogenic and nononcogenic types. "Non-oncogenic HPV" infections included single or multiple infections with only nononcogenic HPV types (6, 11, 26, 40, 42, 53, 54, 55, 61,

62, 64, 67–73, 81–84, IS39, and CP6108) The Pearson's  $\chi^2$  test was used to test for differences for the prevalence of HPV by smoking status and multivariable logistic regression models were used to estimate OR and 95% CI for the association between smoking and HPV prevalence. Two different logistic regression models were used to generate multivariable ORs (mORs). The first model adjusted for only study design variables (i.e., age and country) and the

second model adjusted for the study design variables and a common HIM study list of covariates that are associated with HPV and/or are potential confounders including, where appropriate, ethnicity, circumcision, total number of female partners in the last 3 months, and total number of vaginal sex partners. Interaction was tested with a multiplicative interaction term included in the full multivariable model. All statistical tests were 2-sided. All

**Table 1.** Demographic characteristics by smoking status

Characteristic	Overall (n = 4,054)	Smoking status		
		Never (n = 2,346)	Former (n = 748)	Current (n = 960)
<b>Age</b>				
Mean, (SD) <sup>a</sup>	32.5 (11.1)	30.9 (10.7)	37.3 (12.2)	32.6 (10.1)
<b>Categorical</b>				
18–24	1,227 (30.3)	847 (36.10)	132 (17.7)	248 (25.8)
25–29	621 (15.3)	349 (14.9)	93 (12.4)	179 (18.7)
30–34	610 (15.1)	330 (14.1)	112 (15.0)	168 (17.5)
35–39	538 (13.3)	310 (13.2)	105 (14.0)	123 (12.8)
40–44	421 (10.4)	227 (9.7)	96 (12.8)	98 (10.2)
45–74	637 (15.7)	283 (12.1)	210 (28.1)	144 (15.0)
<b>Country of birth, n (%)<sup>a</sup></b>				
USA	1,338 (33.0)	850 (36.2)	209 (27.9)	279 (29.0)
Mexico	1,394 (34.4)	884 (37.7)	251 (33.6)	259 (27.0)
Brazil	1,322 (32.6)	612 (26.1)	288 (38.5)	422 (44.0)
<b>Race, n (%)<sup>a</sup></b>				
White	1,819 (44.9)	1,097 (46.7)	343 (45.9)	379 (39.5)
Black	633 (15.6)	425 (18.1)	777 (10.3)	131 (13.7)
Asian/Pacific Islander	111 (2.7)	91 (3.8)	8 (1.1)	12 (1.3)
American Indian/Alaska Native	80 (2.0)	47 (2.0)	18 (2.4)	15 (1.6)
Mixed/other/not reported	1,411 (4.4)	686 (29.2)	302 (40.3)	423 (44.1)
<b>Ethnicity, n (%)<sup>a</sup></b>				
Hispanic	1,828 (45.1)	932 (39.7)	373 (49.9)	523 (54.5)
Non-Hispanic	2,191 (54.1)	1,392 (59.3)	371 (49.6)	428 (44.6)
Not Reported/Refused	35 (0.8)	22 (0.94)	4 (0.5)	9 (0.9)
<b>Circumcision, n (%)</b>				
Yes	1,402 (34.6)	842 (35.9)	250 (33.4)	310 (32.3)
No	2,568 (63.3)	1,457 (62.1)	479 (64.0)	632 (65.8)
Partial	84 (2.1)	47 (2.0)	19 (2.5)	18 (1.9)
<b>Lifetime number of vaginal sex partners<sup>a</sup></b>				
0	386 (9.5)	276 (11.8)	36 (4.8)	74 (7.7)
1	325 (8.0)	250 (10.7)	31 (4.1)	44 (4.6)
2–9	1,617 (39.9)	977 (41.7)	291 (38.9)	349 (36.4)
≥10	1,501 (37.0)	732 (31.2)	348 (46.5)	703 (43.8)
Refused	225 (5.6)	111 (4.7)	42 (5.6)	72 (7.5)
<b>Total number of female partners last 3 months, n (%)<sup>a</sup></b>				
None	393 (9.7)	278 (11.9)	38 (5.1)	77 (8.0)
1	2,496 (61.6)	1,388 (59.2)	526 (70.3)	582 (60.6)
2	514 (12.7)	303 (12.9)	85 (11.4)	129 (13.4)
≥3	529 (13.1)	309 (13.2)	81 (10.8)	139 (14.5)
Refused	119 (2.9)	68 (2.9)	18 (2.4)	33 (3.4)

<sup>a</sup>P < 0.05 comparing never, former, current. The  $\chi^2$  test was used to test for differences by smoking status for categorical variables and ANOVA was used for continuous variables (i.e., age).

**Table 2.** Prevalence of HPV infection by smoking status

	Overall <i>n</i> = 4,054	Smoking status			<i>P</i>
		Never <i>n</i> = 2,348	Former <i>n</i> = 748	Current <i>n</i> = 960	
HPV status <sup>a</sup>					
Negative	1,435 (34.9)	861 (36.7)	249 (33.3)	305 (31.8)	
Positive for <sup>b</sup> :					
Any HPV (HPV positive by PCR or genotyping) <sup>c</sup>	2,639 (65.1)	1,485 (63.2)	499 (66.7)	655 (68.2)	0.015
At least 1 oncogenic type <sup>c</sup>	1,226 (30.2)	664 (28.3)	228 (30.5)	334 (34.8)	< 0.001
Only nononcogenic types <sup>c</sup>	845 (20.8)	475 (20.3)	157 (20.9)	213 (22.2)	0.009
Only oncogenic types	476 (11.7)	269 (11.5)	91 (12.2)	116 (12.1)	0.241
Nononcogenic types	1,595 (39.3)	870 (37.1)	294 (39.3)	431 (44.9)	0.001
>1 genotype	1,142 (28.2)	605 (25.8)	208 (27.8)	329 (34.3)	< 0.001
Oncogenic and nononcogenic genotypes	750 (18.5)	395 (16.8)	137 (18.3)	218 (22.7)	< 0.001
At least one genotype	2,071 (51.1)	1,139 (48.6)	385 (51.5)	547 (56.9)	< 0.001
Type 6, 11, 16, or 18	624 (15.4)	333 (14.2)	108 (14.4)	183 (19.1)	0.001
HPV 16 Only	305 (7.5)	161 (6.9)	53 (7.1)	91 (9.5)	0.006

<sup>a</sup>The percentages presented are prevalence using the total number of subjects by smoking status strata as the denominator (e.g., for any HPV positivity among never smokers: 1,485/2,348 = 63.2%).

<sup>b</sup>*P* values were calculated by a  $\chi^2$  test for the distribution of HPV positivity compared with HPV negativity by smoking status.

<sup>c</sup>Any HPV, Oncogenic HPV, and only nononcogenic HPV were used as the dependent variables for the analyses in Table 3–Table 5. Any HPV positivity is defined as HPV positive by PCR or tested positive for at least one genotype. Oncogenic HPV positivity was defined as possessing at least one of the 13 oncogenic types tested for (HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 66), and included men infected with both oncogenic and nononcogenic types. Nononcogenic HPV positivity included single or multiple infections with only nononcogenic HPV types (6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 67–73, 81–84, IS39, and CP6108).

statistical analyses were performed using Stata Intercooled v10.1 (StataCorp.).

## Results

The study population demographic characteristics are presented by smoking status in Table 1. Never smokers (mean age = 30.9; SD 10.7) were younger than former (mean age = 37.3; SD 12.2) and current smokers (mean age = 32.6; SD 10.1; *P* < 0.05). Among never smokers, 36.2% were from the United States, 37.7% from Mexico and 26.1% from Brazil. Compared with current smokers, never smokers had a higher frequency of self-identified Whites (46.7% vs. 39.5%), Blacks (18.1% vs. 13.7%), Asian/Pacific Islanders (3.8% vs. 1.3%), and Non-Hispanics (59.3% vs. 44.6%; *P* < 0.05). Never versus current smokers also reported a slightly higher frequency of circumcision (35.9% vs. 32.3%). Current smokers self-reported a higher frequency of 10 or more lifetime vaginal sex partners (43.8% vs. 31.2%) and a lower frequency of no vaginal sex partners (7.7% vs. 11.8%; *P* < 0.05). Current smokers also reported a higher frequency of total number of female partners in the last 3 months ( $\geq 3$  female partners: 14.5% vs. 13.2%; 2 female partners: 13.4% vs. 12.9%; 1 female partner: 60.6% vs. 59.2%; *P* < 0.05).

Prevalence of any HPV (Table 2) was statistically significantly (*P* = 0.015) lower among never smokers (63.2%)

compared with former (66.7%) and current smokers (68.2%). Similar trends were also observed for prevalence of HPV infection with at least one oncogenic type (*P* < 0.001) and only nononcogenic types (*P* = 0.009). These 3 HPV categories (i.e., "Any HPV," "Oncogenic HPV," and "Only nononcogenic HPV") were used as the dependent variables for the analyses in Table 3–Table 5. In addition, the prevalence of infection for the other HPV categories in Table 2 were all statistically significantly lower for never smokers except for only oncogenic types which revealed no statistically significant difference (*P* = 0.241).

To determine whether there were differences (i.e., potential confounding) in HPV positivity with increasing smoking intensity, we assessed the prevalence of HPV infection across tertiles of cigarettes smoked/day, years smoked, and pack-years smoked for current and former smokers. Among former smokers, there was no statistically significant difference in the prevalence of HPV infection by the tertile distributions of cigarettes smoked per day and pack-years smoked (Table 3). However, prevalence of any HPV infection was higher for the third tertile of years smoked versus the first tertile (73.3% vs. 62.9%; *P* = 0.025), as was prevalence of only nononcogenic HPV (23.9% vs. 21.7%; *P* = 0.041). We also assessed mean number of cigarettes smoked per day and mean pack-years smoked (i) between former and current smokers for each HPV category (i.e., "Any HPV," "Oncogenic HPV,"

**Table 3.** Prevalence of HPV infection by smoking status and tertiles<sup>b</sup> of smoking characteristics

HPV status	Former smokers				Current smokers			
	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	P <sup>a</sup>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	P <sup>a</sup>
Total	n = 255	n = 258	n = 215		n = 320	n = 352	n = 275	
Tertiles cigarettes smoked/day								
Negative	97 (38.0%)	86 (37.7%)	62 (28.8%)		107 (33.4%)	117 (33.2%)	79 (28.7%)	
Any HPV	158 (62.0%)	172 (75.4%)	153 (71.2%)	0.108	213 (66.6%)	235 (66.8%)	196 (71.3%)	0.386
Oncogenic HPV	73 (28.6%)	80 (35.1%)	68 (31.6%)	0.265	103 (32.2%)	128 (36.4%)	96 (34.9%)	0.520
Only nononcogenic HPV	48 (18.8%)	53 (21.5%)	50 (23.3%)	0.168	74 (23.1%)	68 (19.3%)	67 (24.4%)	0.244
Total	n = 240	n = 249	n = 243		n = 142	n = 321	n = 479	
Tertiles of years smoked								
Negative	89 (37.1%)	91 (36.5%)	65 (26.7%)		50 (35.2%)	96 (29.9%)	156 (32.6%)	
Any HPV	151 (62.9%)	158 (63.5%)	178 (73.3%)	0.025	92 (64.8%)	225 (70.1%)	323 (67.4%)	0.500
Oncogenic HPV	67 (27.9%)	74 (29.7%)	81 (33.3%)	0.064	46 (32.4%)	124 (38.6%)	155 (32.4%)	0.235
Only nononcogenic HPV	52 (21.7%)	43 (17.3%)	58 (23.9%)	0.041	25 (14.6%)	69 (21.5%)	114 (23.8%)	0.363
Total	n = 262	n = 233	n = 222		n = 215	n = 359	n = 362	
Tertiles of pack-years								
Negative	95 (36.3%)	85 (36.5%)	61 (27.5%)		75 (34.9%)	121 (33.7%)	104 (28.7%)	
Any HPV	167 (63.7%)	148 (63.5%)	161 (72.5%)	0.066	140 (65.1%)	217 (66.3%)	225 (71.3%)	0.497
Oncogenic HPV	79 (30.2%)	68 (29.2%)	72 (32.4%)	0.199	71 (33.0%)	141 (34.5%)	111 (35.4%)	0.602
Only nononcogenic HPV	51 (19.5%)	45 (19.3%)	52 (23.4%)	0.117	44 (31.0%)	85 (26.5%)	77 (16.1%)	0.617

<sup>a</sup>P values were calculated by a  $\chi^2$  test for the distribution comparing each HPV positivity category (i.e., "Any HPV," "Oncogenic HPV," and "Only nononcogenic HPV") to HPV negativity.

<sup>b</sup>Tertiles were defined by overall smoking characteristic distributions by smoking status.

and "Only nononcogenic HPV"), and (ii) among former and current smokers by HPV status (compared with participants who were HPV negative) and found no statistically significant differences (data not shown).

When we assessed the association between smoking and HPV infection (Table 4), we found that current smokers were statistically significantly associated with an increased risk for any HPV (OR = 1.19; 95% CI: 1.01–1.41) and oncogenic HPV (OR = 1.24; 95% CI: 1.05–1.47) after adjusting for age, race, ethnicity, country, circumcision, total number of female partners in the last 3 months, and total number of vaginal sex partners. Compared with never smokers, current smokers who smoked greater than 5 pack-years exhibited an increased risk of oncogenic HPV (OR = 1.29; 95% CI: 1.02–1.64). We found no statistically significant association between smoking and nononcogenic HPV infection or among men who smoked less than 5 pack-years.

We assessed the interplay of sexual activity, smoking, and risk of HPV infection (Table 5) and found that current smokers with the fewest number of partners (1–9 partners) were at increased risk of any HPV infection (OR = 1.35; 95% CI: 1.05–1.73) and oncogenic HPV infection (OR = 1.46; 95% CI: 1.11–1.92) after adjusting for age, race, ethnicity, country, circumcision, total number of female partners in the last 3 months, and total number of vaginal sex partners. Among men with zero lifetime

partners, elevated ORs that were not statistically significant were observed for any and oncogenic HPV infection. As noted in Table 4, we found no statistically significant associations for only nononcogenic HPV infection for any of the strata. We also found that men with 10 or more lifetime partners consistently exhibited ORs near the null. We also stratified number of partners by more narrow categories (i.e., 1–3; 4–6, 7–9; 10–49, and  $\geq 50$ ), but found no appreciable differences in the results (data not shown).

Finally, we also analyzed the data by country, sexual orientation, and circumcision and found that the results were relatively consistent for: all 3 countries, by sexual orientation [i.e., no sex, men who have sex with women and men (MSWM), and men who have sex with men (MSM), and among noncircumcised men (data not shown)]. However, because of the reduced sample size the point estimates were not statistically significant for the 3 countries and among noncircumcised men. Moreover, the number of MSM for each stratum was quite small which yielded imprecise and uninterpretable point estimates.

## Discussion

In this multinational cohort study of HPV in men, our analyses suggest that current smoking was associated with an increased risk of any HPV infection and oncogenic

Table 4. Risk of HPV infection by smoking characteristics

	Any HPV		Oncogenic HPV		Only nononcogenic HPV	
	mOR (95% CI) <sup>a</sup>	mOR (95% CI) <sup>b</sup>	mOR (95% CI) <sup>a</sup>	mOR (95% CI) <sup>b</sup>	mOR (95% CI) <sup>a</sup>	mOR (95% CI) <sup>b</sup>
Smoking status						
Never smoker	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
Former smoker	1.14 (0.95–1.37)	1.07 (0.88–1.29)	1.14 (0.95–1.38)	1.06 (0.87–1.29)	0.96 (0.77–1.18)	0.95 (0.77–1.18)
Current smoker	1.32 (1.12–1.56)	1.19 (1.01–1.41)	1.42 (1.21–1.68)	1.24 (1.05–1.47)	1.10 (0.91–1.33)	1.05 (0.87–1.27)
Pack-years smoked						
Never smokers	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
<5 pack-years (current smokers)	1.25 (1.01–1.54)	1.13 (0.92–1.40)	1.35 (1.09–1.67)	1.19 (0.96–1.48)	1.10 (0.86–1.40)	1.05 (0.82–1.35)
≥5 pack-years (current smokers)	1.39 (1.09–1.77)	1.23 (0.96–1.57)	1.52 (1.21–1.91)	1.29 (1.02–1.64)	1.11 (0.86–1.44)	1.06 (0.82–1.38)
Never smokers	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
<5 pack-years (former smokers)	1.06 (0.86–1.31)	0.99 (0.80–1.23)	1.10 (0.88–1.38)	1.04 (0.83–1.31)	0.95 (0.74–1.23)	0.94 (0.73–1.21)
≥5 pack-years (former smokers)	1.30 (0.97–1.75)	1.16 (0.85–1.57)	1.20 (0.90–1.60)	1.08 (0.81–1.46)	0.92 (0.67–1.26)	0.89 (0.65–1.24)

<sup>a</sup>mOR = multivariable OR adjusted for age and country.<sup>b</sup>mOR = multivariable OR adjusted for age, race, ethnicity, country, circumcision, total number of female partners in the last 3 months, and total number of vaginal sex partners.

HPV infection. However, the observed association with smoking was limited to men reporting fewer lifetime female sexual partners.

Limited data exist on the association between HPV infection and smoking in men. Our results are generally consistent with prior findings in women that cigarette smoking is associated with HPV load (39), HPV prevalence (8, 12–15), incidence (11, 16), and persistence (9, 10). At present, it is unclear how smoking may influence HPV infection in men, but many possible mechanisms exist. For instance, laboratory studies have shown that smoking increases cellular proliferation and metaplasia in various tissues and cell types (23, 29, 31, 33, 34), which in turn could result in an increase in replication or production of HPV due to smoking-induced cell proliferation. Constituents of cigarette smoke have also been shown to modify the function of immune cells (24, 25). For example, acrolein, an aldehyde found in tobacco smoke, affects neutrophil function (20), causes DNA damage (19) and has been shown to suppress resistance to pulmonary infections (27). Smoking could also potentially increase viral load by weakening the cellular immune response because previous studies have shown that smoking has deleterious effects on both systemic and local immunity (18, 26, 30, 32). Smoking results in the recruitment of inflammatory cells and subsequent release of proinflammatory cytokines, chemotactic factors, oxygen radicals, and proteases which alter the function of immune cells (28). Nicotine, the main compound responsible for the dependence-forming properties of smoking, has also been shown to be immunosuppressive in both animals (21) and in humans (22).

Although in our analysis current smoking was associated with a statistically significantly elevated risk of any HPV infection (OR = 1.19), it is likely that this effect is largely driven by the elevated risk of oncogenic HPV infection among current smokers (OR = 1.24). Consistent with the observation that current smokers may be at an increased risk of oncogenic HPV, we also found a statistically significant increased risk of oncogenic HPV among current smokers with a 5 or more pack-year history (OR = 1.29). No statistically significant effects were found among former smokers or among men who smoked less than 5 pack-years, possibly due to the immunomodulatory properties observed among individuals who are actively exposed to cigarette smoke (18, 26, 30, 32). We also observed a novel interplay between smoking and number of sexual partners revealing that men with fewer or no sexual partners were at an increased risk for HPV infection versus men with a greater number of sexual partners. Specifically, men with zero or less than 9 lifetime sexual partners exhibited modest elevated risks for any and oncogenic HPV infection; however, the ORs were only statistically significant for the strata of 1 to 9 sexual partners. Moreover, no statistically significant effects were found among men with greater than 10 lifetime sexual partners. Increased sexual activity may result in higher HPV exposure which would then mask the influence of

**Table 5.** Risk of HPV infection by sexual activity and smoking status

	Lifetime number of female sex partners						<i>P</i> <sub>for interaction</sub>
	0		1-9		≥ 10		
	mOR (95% CI) <sup>a</sup>	mOR (95% CI) <sup>b</sup>	mOR (95% CI) <sup>a</sup>	mOR (95% CI) <sup>b</sup>	mOR (95% CI) <sup>a</sup>	mOR (95% CI) <sup>b</sup>	
<b>Any HPV by number of female partners</b>							
Never smokers	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	
Former smokers	1.32 (0.61-2.85)	1.48 (0.65-3.34)	1.11 (0.85-1.43)	1.08 (0.83-1.40)	0.87 (0.64-1.17)	0.83 (0.61-1.13)	0.123
Current smoker	1.29 (0.76-2.21)	1.31 (0.76-2.27)	1.38 (1.08-1.77)	1.35 (1.05-1.73)	0.94 (0.71-1.25)	0.93 (0.98-1.23)	0.201
<b>Oncogenic HPV by number of female partners</b>							
Never smokers	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	
Former smokers	1.40 (0.62-3.11)	1.51 (0.65-3.48)	1.14 (0.84-1.55)	1.14 (0.84-1.55)	0.93 (0.71-1.22)	0.91 (0.69-1.20)	0.505
Current smoker	1.31 (0.72-2.39)	1.34 (0.72-2.47)	1.46 (1.10-1.92)	1.46 (1.11-1.92)	1.05 (0.82-1.35)	1.04 (0.81-1.34)	0.292
<b>Only nononcogenic HPV</b>							
Never smokers	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	
Former smokers	1.10 (0.48-2.70)	1.14 (0.45-2.84)	0.81 (0.58-1.14)	0.80 (0.57-1.12)	0.89 (0.67-1.22)	0.89 (0.65-1.21)	0.801
Current smoker	0.77 (0.37-1.64)	0.80 (0.38-1.70)	1.14 (0.84-1.54)	1.11 (0.82-1.51)	1.00 (0.76-1.33)	1.00 (0.76-1.33)	0.684

<sup>a</sup>mOR = multivariable OR adjusted for age and country.<sup>b</sup>mOR = multivariable OR adjusted for age, race, ethnicity, country, and circumcision.

cigarette smoking. However, this is merely speculative and, moreover, none of the interaction tests were statistically significant. Thus, this finding warrants further analysis in other cohorts and longitudinal analyses.

The major strengths of this study are the inclusion of a well-characterized international cohort of men of a wide age range (i.e., 18-70 years of age), and the availability of extensive and previously validated participant information (40). Another strength of the study is the large sample size of the baseline cohort, although stratification by smoking and sexual behavior did result in smaller subgroups in the present analysis. There was little evidence of confounding for the oncogenic HPV results as shown by the relative consistency between the 2 multivariable models for each analysis. One model included only the study design variables and the other model included both the study design variables and potential confounders. None of the ORs between the 2 models differed by more than 10% for the oncogenic HPV results. Hence, presenting data from the more inclusive multivariable model is likely more conservative. In addition, the data in Table 3 suggest that there was no evidence that smoking intensity was confounding the results among current smokers because there were no differences in prevalence of HPV infection with increasing smoking intensity (cigarettes smoked/day, years smoked, and pack-years) among current smokers. Yet, we do acknowledge that we cannot account for bias due to unmeasured or unknown confounding cannot be accounted for. Sexual behavior is potentially an important confounder in the association between smoking and HPV positivity. Although we accounted for potential confounding by adjusting for self-reported sexual behavior and stratified by number of female partners, residual confounding still may exist which could potentially inflate the observed point estimates. Hence, our results should be interpreted with caution. Although this is a baseline cross-sectional analysis, these data are important and novel because there is limited information on the association between smoking and HPV in men. Future longitudinal analyses will be performed to assess whether smoking influences HPV acquisition and clearance. We do acknowledge that the cohort may not be a representative sample of the general male population of the participating countries, which limits the generalizability of our findings.

The biological role that smoking plays in HPV infection in men remains understudied, and limited data exist on the association between smoking and HPV infection. This analysis thus provides important data on the interplay between smoking, sexual activity, and men's risk of HPV infection in men. Overall, these results showed that current smokers are associated with an increased risk oncogenic HPV infection.

#### Disclosure of Potential Conflicts of Interest

A. R. Giuliano receives support from Merck, is a member of the Merck Young Women's Advisory Board, and serves on the speakers'

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