

HUMAN PAPILLOMAVIRUS INFECTION AS A RISK FACTOR FOR SQUAMOUS-CELL CARCINOMA OF THE HEAD AND NECK

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ABSTRACT

Background Oncogenic human papillomaviruses (HPVs), especially HPV type 16 (HPV-16), cause anogenital epithelial cancers and are suspected of causing epithelial cancers of the head and neck.

Methods To examine the relation between head and neck cancers and HPVs, we performed a nested case-control study within a joint Nordic cohort in which serum samples were collected from almost 900,000 subjects. Samples collected at enrollment from 292 persons in whom squamous-cell carcinoma of the head and neck developed, on average, 9.4 years after enrollment and from 1568 matched controls were analyzed for antibodies against HPV-16, HPV-18, HPV-33, and HPV-73 and for cotinine levels as a marker of smoking habits. Polymerase-chain-reaction (PCR) analyses for HPV DNA were performed in tumor tissue from 160 of the study patients with cancer.

Results After adjustment for cotinine levels, the odds ratio for squamous-cell carcinoma of the head and neck in subjects who were seropositive for HPV-16 was 2.2 (95 percent confidence interval, 1.4 to 3.4). No increased risk was observed for other HPV types. Fifty percent of oropharyngeal and 14 percent of tongue cancers contained HPV-16 DNA, according to PCR analysis.

Conclusions HPV-16 infection may be a risk factor for squamous-cell carcinoma of the head and neck. (N Engl J Med 2001;344:1125-31.)

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O NCOGENIC human papillomaviruses (HPVs) are a major cause of anogenital cancers.¹ HPV has also been implicated in head and neck cancer, because viral DNA, mostly of HPV type 16 (HPV-16), has been found in tumor tissue.^{1,2} However, the results of case series and case-control studies are not consistent.¹⁻⁷ The finding of an association in case-control studies in which tissue was collected after the diagnosis of the cancer may only mean that the disease activated the virus or influenced the sampling and detection of the virus. For epidemiologic evaluation of causality, studies based on samples from healthy persons in whom the disease later develops are essential.

HPV infection is commonly identified by detecting viral DNA in cells or tissues, but because HPV infections are focal, there are sampling errors associ-

ated with this method, especially in asymptomatic subjects. And because most HPV infections are transient, the absence of HPV DNA does not rule out previous exposure.^{8,9} Antibodies to HPV capsid antigens are reliable markers of past or present HPV infection,^{10,11} and seroepidemiologic methods have been used in prospective studies that linked HPV-16 infection to cervical¹² and anogenital¹³ cancers. Our goal was to evaluate HPV infection as a risk factor for the development of squamous-cell carcinoma of the head and neck.

METHODS

Subjects and Study Design

Almost 900,000 residents of Norway, Finland, and Sweden have donated serum samples to the four serum banks participating in the study (additional information is available with the full text of the article at <http://www.nejm.org>).

Persons who had donated serum at least one month before a diagnosis of a head or neck cancer were identified by linkage of serum-bank files with the national cancer registries in Norway, Finland, and Sweden. Reporting of new cases of cancer is compulsory in these three countries, and reliance on multiple data sources ensures that the cancer registries are almost 100 percent complete.¹⁴

Head and neck sites were defined according to the following codes of the *International Classification of Diseases, Seventh Revision*¹⁵: 140 (vermillion border of the lips), 141 (tongue), 143 (floor of mouth), 144 (oral cavity, not otherwise specified), 145 (oropharynx), 146 (nasopharynx), 147 (hypopharynx), 148 (pharynx, not otherwise specified), 160 (nose and paranasal sinuses), and 161 (larynx).

From the creation of the serum banks through 1997, 301 invasive squamous-cell carcinomas and 8 carcinomas of the head and neck (not otherwise specified) were registered. Reevaluation of pathological and clinical features led to the exclusion of four cases because the histologic diagnosis was uncertain and two cases because their true anatomical location was outside the sites designat-

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ed for the study. Of the eight cases of carcinoma not otherwise specified, two cases reclassified as squamous-cell carcinoma were included, and the other six unspecified carcinomas were excluded. In five cases, serum samples were not available. The characteristics of the remaining 292 patients are given in Table 1. If more than one prediagnostic serum sample was available, the first (oldest) sample was chosen. The mean time between enrollment and diagnosis was 9.4 years (range, 2 months to 19.3 years).

For each patient, five (Norway and Sweden) or seven (Finland) matched control subjects were selected. The controls were alive and free of head and neck cancer at the time the corresponding patient received a diagnosis of cancer. The matching variables were sex, age at the diagnosis of cancer in the corresponding patient (within two years), and length of serum storage (within two months). Matching of patients and controls was performed entirely within each cohort (serum bank) to ensure that differences between the cohorts did not affect the validity of the study. In Norway, the patients and controls were also matched according to county of residence. If five matched control subjects per patient could not be found, the matching criteria for age and serum storage time were expanded stepwise by one year of age and two months of serum storage. The mean difference in age between patients and controls was 0.9 year, and the maximal difference was 4 years. The mean difference in serum storage time was 0.8 month, and the maximal difference was 6 months. After the exclusion of 22 eligible controls for whom serum samples were not available, the control group contained 1568 persons. There were at least four matched controls for each patient. Diagnostic histologic specimens from 228 of 292 patients were received from pathological laboratories for histopathological review and polymerase-chain-reaction (PCR) analysis.

Laboratory Methods

Antibodies against HPV were detected by the standard enzyme-linked immunosorbent assay, with the use of baculovirus-expressed capsids containing both the L1 and the L2 proteins (major onco-genic HPV-16, HPV-18, and HPV-33) or only L1 (HPV-73). HPV-73 has been cloned from an esophageal carcinoma.¹⁶ Disrupted capsids of bovine papillomavirus served as a negative control. The cutoff levels used to assign seropositivity from continuous absorbance values were preassigned and, relative to internal standard serum, were the same as those used in previous studies.^{5,12,13,17,18} For the different viruses, the interassay coefficients of variation ranged from 17.8 percent to 33.8 percent, and the intraassay coefficients of variation ranged from 5.3 percent to 10.4 percent.

Serum cotinine, a biochemical marker of exposure to tobacco smoke,¹⁹ was measured by a quantitative competitive enzyme immunoassay that used microtiter plates coated with anticotinine antibodies and detection with a cotinine-horseradish peroxidase conjugate (STC Technologies, Bethlehem, Pa.). On the basis of previous reports,^{19,21} prospectively chosen cutoff levels of serum cotinine were used to identify nonsmokers and those "passively exposed to smoke" (0 to 19.99 ng of cotinine per milliliter), "light to moderate smokers" (20.00 to 224.99 ng of cotinine per milliliter), and "heavier smokers" (≥ 225.00 ng of cotinine per milliliter).

Formalin-fixed, paraffin-embedded tissues were examined for HPV DNA by PCR assay. The quality of DNA was tested by amplification of HLA-DQA1 with the primers GH26 and GH27.²² All samples from patients with cancer that were positive for these primers were examined for HPV DNA with the L1 consensus primers GP5+ and GP6+²³ and the E1 consensus primers CpI and

TABLE 1. CHARACTERISTICS OF THE PATIENTS WITH HEAD AND NECK CANCER, ACCORDING TO COHORT.

CHARACTERISTIC	JANUS SERUM BANK (N=214)	HELSINKI HEART STUDY (N=41)	FINNISH MATERNITY COHORT (N=24)	NORTHERN SWEDEN HEALTH AND DISEASE STUDY (N=13)	ALL COHORTS (N=292)
	number (percent)				
Sex					
Male	185 (86)	41 (100)	0	10 (77)	236 (81)
Female	29 (14)	0	24 (100)	3 (23)	56 (19)
Age at diagnosis					
0-39 yr	5 (2)	0	14 (58)	1 (8)	20 (7)
40-49 yr	48 (22)	1 (2)	10 (42)	1 (8)	60 (21)
50-59 yr	103 (48)	24 (59)	0	5 (38)	132 (45)
≥ 60 yr	58 (27)	16 (39)	0	6 (46)	80 (27)
Time between enrollment and diagnosis					
2 mo-4 yr	50 (23)	12 (29)	9 (38)	8 (62)	79 (27)
5-14 yr	111 (52)	29 (71)	15 (62)	5 (38)	160 (55)
≥ 15 yr	53 (25)	0	0	0	53 (18)
Site of head and neck cancer*					
Lips (code 140)	41 (19)	11 (27)	2 (8)	3 (23)	57 (20)
Tongue (code 141)	44 (21)	2 (5)	9 (38)	2 (15)	57 (20)
Floor of mouth (code 143)	17 (8)	2 (5)	4 (17)	0	23 (8)
Oral cavity, not otherwise specified (code 144)	12 (6)	3 (7)	2 (8)	2 (15)	19 (7)
Oropharynx (code 145)	18 (8)	1 (2)	4 (17)	3 (23)	26 (9)
Nasopharynx (code 146)	5 (2)	3 (7)	2 (8)	0	10 (3)
Hypopharynx (code 147)	13 (6)	1 (2)	0	2 (15)	16 (5)
Pharynx, not otherwise specified (code 148)	1 (<1)	0	0	0	1 (<1)
Nose and paranasal sinuses (code 160)	5 (2)	0	1 (4)	1 (8)	7 (2)
Larynx (code 161)	58 (27)	18 (44)	0	0	76 (26)

*The numbers in parentheses refer to the codes of the *International Classification of Diseases, Seventh Revision*.¹⁵

CpIIG,²⁴ as previously described.²⁵ Empty paraffin-block sections were cut between samples from patients with cancer and used as contamination controls for each PCR assay. HPV-DNA-positive samples were tested with E6 and E7 type-specific primers for HPV-6, HPV-11, HPV-16, HPV-18, and HPV-33.²⁵⁻²⁷ All samples that were negative for HPV DNA were also tested with primers specific for HPV-16.

All laboratory analyses were performed with masked samples, and then the data were submitted to the Cancer Registry of Norway for decoding and statistical analysis.

Statistical Analysis

Odds ratios and their 95 percent confidence intervals were derived from conditional logistic-regression models with the Epicure program.²⁸ The logistic-regression analyses reflected the three matching variables of sex, age, and length of serum storage. Likelihood-ratio tests evaluated variables in the model, including a test of homogeneity in odds ratios. Pearson's correlation coefficient estimated the correlation between variables. Fisher's exact test was used to test for equity between proportions. A two-tailed P value of less than 0.05 was considered to indicate statistical significance.

RESULTS

The prevalence of seropositivity for HPV-16 was almost twice as high among patients with head and neck cancer as among controls (12 percent vs. 7 percent) (Table 2). For HPV-18, HPV-33, and HPV-73, the seroprevalence was similar in both groups. After adjustment for cotinine levels, the risk of squamous-cell carcinoma of the head and neck was significantly associated with HPV-16 seropositivity (odds ratio, 2.2; 95 percent confidence interval, 1.4 to 3.4), whereas no significantly increased risks were observed for HPV-18, HPV-33, or HPV-73 (Table 2). The crude odds ratios were similar to the adjusted values, and adjustment for the cotinine level as a continuous variable (i.e., the use of all continuous data, without a cutoff

level) also did not substantially change the HPV-16-associated risk estimate. Analysis with the level of antibodies against HPV as a continuous variable likewise revealed HPV-16 seropositivity as a risk factor (P<0.001), but none of the other HPV types were incriminated (P>0.5 for any other HPV type); this result indicates that the preassigned cutoff levels used in the dichotomous analyses were representative. The cotinine level and seropositivity for HPV-16 were not significantly correlated (r = -0.04, P = 0.12). As expected, the odds ratio for squamous-cell carcinoma of the head and neck increased with increasing levels of serum cotinine (Table 2).

Only a few patients had intermediate serum cotinine values (20.00 to 224.99 ng per milliliter). Therefore, stratified risk analyses were performed according to epithelial origin and anatomical site with the cotinine level as a dichotomous variable (smokers vs. nonsmokers). For lip cancer, which according to its definition in the Nordic cancer registries develops from the skin of the vermilion border, no significantly increased risk was seen in association with seropositivity for HPV-16 (Table 3). The same was true for cancers of the nose and paranasal sinuses and cancers of the nasopharynx, which originate from respiratory epithelium, but the numbers of cases were small and thus the confidence intervals were wide (Table 3). For all the other sites, which are lined by mucosal stratified squamous-cell epithelium (and which represented 73 percent of all patients), the adjusted odds ratio was increased (odds ratio, 2.6; 95 percent confidence interval, 1.7 to 4.2) (Table 3).

There was significant heterogeneity in the odds ratios across anatomical sites (P<0.001). Significantly

TABLE 2. ODDS RATIOS FOR HEAD AND NECK CANCER ASSOCIATED WITH SEROPOSITIVITY FOR HUMAN PAPILLOMAVIRUS (HPV) AND WITH TOBACCO USE AS MEASURED BY COTININE LEVEL.*

EXPOSURE	No. (%) OF PATIENTS	No. (%) OF CONTROLS	CRUDE ODDS RATIO (95% CI)	P VALUE	ADJUSTED ODDS RATIO (95% CI)	P VALUE
Seropositivity						
HPV-16	35 (12)	102 (7)	2.1 (1.4-3.2)	<0.001	2.2 (1.4-3.4)†	<0.001
HPV-18	17 (6)	101 (6)	1.0 (0.6-1.6)	0.87	1.0 (0.6-1.8)†	0.91
HPV-33	22 (8)	154 (10)	0.8 (0.5-1.3)	0.33	0.8 (0.5-1.3)†	0.27
HPV-73	14 (5)	111 (7)	0.7 (0.4-1.2)	0.19	0.6 (0.4-1.2)†	0.13
Cotinine						
0-19.99 ng/ml‡	63 (22)	724 (46)	1.0	<0.001	1.0	<0.001
20.00-224.99 ng/ml	23 (8)	184 (12)	1.5 (0.9-2.5)		1.5 (0.9-2.5)§	
≥225.00 ng/ml	206 (71)	658 (42)	4.0 (2.9-5.5)		4.0 (2.9-5.6)§	

*CI denotes confidence interval. Cotinine values were missing for two controls.

†The odds ratio was adjusted for three levels of cotinine.

‡Subjects in this category served as the reference group.

§The odds ratio was adjusted for HPV-16 seropositivity.

TABLE 3. ODDS RATIOS FOR HEAD AND NECK CANCER ASSOCIATED WITH SEROPOSITIVITY FOR HUMAN PAPILLOMAVIRUS TYPE 16, ACCORDING TO EPITHELIAL TYPE.*

EPITHELIAL TYPE (SITE)†	SEROPOSITIVE PATIENTS	SEROPOSITIVE CONTROLS	CRUDE ODDS RATIO (95% CI)	ADJUSTED ODDS RATIO (95% CI)‡
	no./total no. (%)			
Skin (code 140)	2/57 (4)	21/307 (7)	0.5 (0.1–2.4)	0.5 (0.1–2.1)
Respiratory epithelium (codes 146, 160)	2/17 (12)	5/96 (5)	2.1 (0.4–11.1)	2.8 (0.5–15.9)
Mucosal stratified squamous epithelium (codes 141, 143, 144, 145, 147, 148, 161)	31/218 (14)	76/1165 (7)	2.5 (1.6–4.0)	2.6 (1.7–4.2)

*CI denotes confidence interval.

†The numbers in parentheses refer to the codes of the *International Classification of Diseases, Seventh Revision*.¹⁵

‡The odds ratios were adjusted for two levels of cotinine (nonsmoker, <20.00 ng per milliliter; smoker, ≥20.00 ng per milliliter).

elevated odds ratios were detected for cancers of the tongue (adjusted odds ratio, 2.8; 95 percent confidence interval, 1.2 to 6.6) and oropharynx (adjusted odds ratio, 14.4; 95 percent confidence interval, 3.6 to 58.1) (Table 4). Most of the oropharyngeal cancers (21 of 26) originated from the tonsils. The adjusted odds ratio for tonsillar cancer alone was 10.2 (95 percent confidence interval, 2.4 to 42.9). Seventeen of 57 tongue cancers originated from the base of the tongue. The adjusted odds ratio for cancer of the base of the tongue alone was 20.7 (95 percent confidence interval, 2.7 to 160.1).

The HPV-16–associated risk of head and neck cancer of mucosal stratified squamous-cell epithelium was not significantly different in men and women; the odds ratios were 2.3 (95 percent confidence interval, 1.3 to 4.0) for men and 3.5 (95 percent confidence interval, 1.5 to 7.7) for women ($P=0.33$). There was no significant difference in the HPV-16–associated risks with different lengths of time between serum sampling and diagnosis ($P=0.39$) (Table 5).

DNA was successfully extracted from 160 of 228 tumor specimens. Fifteen of the 160 tumor specimens (9 percent) were positive for HPV-16 DNA according to PCR (Table 4). The corresponding numbers for HPV-6, HPV-11, HPV-18, and HPV-33 were one, two, zero, and one, respectively. Fourteen specimens contained DNA of other HPV types. Most of the tumors positive for HPV-16 DNA were oropharyngeal tumors (Table 4). Detection of HPV-16 DNA in the tumors correlated with prediagnostic seropositivity for HPV-16: 8 of 15 cases that were positive for HPV-16 DNA had prediagnostic seropositivity for HPV-16, but only 16 of 145 cases that were negative for HPV-16 DNA were seropositive before the diagnosis ($P<0.001$). The risk of having a head and neck cancer that contained HPV-16 DNA in HPV-16–

seropositive subjects was significant (odds ratio, 37.5; 95 percent confidence interval, 4.0 to 348.8), whereas the risk of cancers that did not carry the viral genome was much lower (odds ratio, 2.1; 95 percent confidence interval, 1.1 to 3.8).

DISCUSSION

Most studies of HPV in head and neck cancer are case series, with or without a comparison group.^{1,2} In several studies, tonsillar and oropharyngeal carcinomas contained HPV DNA more commonly than cancers at other head and neck sites,^{3,4,29–31} in line with findings that patients with a history of anogenital cancer have 4.3 times the risk for tonsillar cancer of the general population.³² The squamous epithelium lining Waldeyer's tonsillar ring might be particularly susceptible to HPV owing to facilitated viral access to basal mucosal cells in the tonsillar crypts.^{30,32} These case series cannot, however, be used to assess the role of HPV infection in the subsequent risk of head and neck cancer.

The viruses we examined primarily infect the anogenital tract. Since a serologic assay is not site-specific, it could be argued that infections outside the head and neck might have influenced our risk estimates. We believe, however, that the risk associated with seropositivity was largely attributable to infection at the site of the tumor, because the odds ratio was significantly higher for tumors that were positive for HPV-16 DNA (37.5) than for those that were negative (2.1). This conclusion must be tempered by the relatively small number of tumors (8 of 15 of the former type and 16 of 145 of the latter type), which is reflected in the wide confidence interval (4.0 to 348.8).

How HPV infects the upper respiratory tract is not firmly established, but epidemiologic evidence suggests sexual transmission. In three case–control studies, pa-

TABLE 4. ODDS RATIOS FOR HEAD AND NECK CANCER ASSOCIATED WITH SEROPOSITIVITY FOR HUMAN PAPILLOMAVIRUS TYPE 16 (HPV-16), ACCORDING TO ANATOMICAL SITE, IN COMPARISON WITH THE PREVALENCE OF VIRAL DNA IN TUMOR TISSUE.*

SITE†	SEROPOSITIVE PATIENTS	SEROPOSITIVE CONTROLS	CRUDE ODDS RATIO (95% CI)	ADJUSTED ODDS RATIO (95% CI)‡	PATIENTS POSITIVE FOR HPV-16 DNA§
	no./total no. (%)				no./total no. (%)
Lips (code 140)	2/57 (4)	21/307 (7)	0.5 (0.1–2.4)	0.5 (0.1–2.1)	0/32 (0)
Tongue (code 141)	9/57 (16)	22/302 (7)	2.7 (1.2–6.4)	2.8 (1.2–6.6)	4/29 (14)
Floor of mouth (code 143)	0/23 (0)	15/125 (12)	—	—	0/15 (0)
Oral cavity, not otherwise specified (code 144)	2/19 (11)	2/104 (2)	5.4 (0.8–38.8)	3.6 (0.5–26.3)	0/15 (0)
Oropharynx (code 145)	10/26 (38)	14/137 (10)	8.6 (2.6–28.5)	14.4 (3.6–58.1)	9/18 (50)
Nasopharynx (code 146)	0/10 (0)	2/60 (3)	—	—	1/7 (14)
Hypopharynx (code 147)	0/16 (0)	3/81 (4)	—	—	0/8 (0)
Nose and paranasal sinuses (code 160)	2/7 (29)	3/36 (8)	3.5 (0.6–20.7)	3.4 (0.6–20.8)	0/4 (0)
Larynx (code 161)	9/76 (12)	20/411 (5)	2.5 (1.1–5.8)	2.4 (1.0–5.6)	1/32 (3)
All sites	35/292 (12)	102/1568 (7)	2.1 (1.4–3.2)	2.1 (1.4–3.2)¶	15/160 (9)

*CI denotes confidence interval.

†The numbers in parentheses refer to the codes of the *International Classification of Diseases, Seventh Revision*.¹⁵ Site 148 (pharynx, not otherwise specified) was the location of only one cancer and is not listed.

‡The odds ratios were adjusted for two levels of cotinine (nonsmoker, <20.00 ng per milliliter; smoker, ≥20.00 ng per milliliter).

§Tumor tissue was taken from 160 patients.

¶The difference between this estimate and the one given in Table 2 (2.1 vs. 2.2) is a consequence of the use of two as compared with three levels of cotinine in the adjustment procedure.

tients with oral cancer had had more sexual partners than controls, although the numbers of patients and controls who had ever had oral–genital sexual contact were not significantly different.^{4,6,7} One of these studies⁴ found that the associations with a higher lifetime number of sexual partners and with a total of more than four partners with whom the subjects engaged in oral sex was stronger for patients with tumors positive for HPV-16 DNA than for those whose tumors did not contain HPV-16 DNA.

Antibodies against HPV have high specificity for sexually transmitted types of HPV, since seropositivity is rare among virginal or monogamous women.³³ However, the sensitivity of the serologic assay is suboptimal. Validation studies have concluded that only about 50 to 70 percent of genitally infected women (as determined by PCR) will seroconvert.^{10,11,34} Non-differential misclassification of exposure due to moderate sensitivity, however, probably had little effect on our risk estimates (50 percent sensitivity was predicted to result in estimates less than 10 percent conservatively biased). Neither our study nor a previous population-based study¹³ found any significant sex-related differences in the risk of cancer associated with the presence of anti-HPV antibodies.

A high level of alcohol consumption, both alone and in combination with smoking, is a risk factor for

TABLE 5. ODDS RATIOS FOR HEAD AND NECK CANCER ORIGINATING FROM MUCOSAL STRATIFIED SQUAMOUS EPITHELIUM ASSOCIATED WITH SEROPOSITIVITY FOR HUMAN PAPILLOMAVIRUS TYPE 16, ACCORDING TO THE TIME BETWEEN SERUM SAMPLING AND DIAGNOSIS.*

TIME	TOTAL NO. OF PATIENTS	NO. (%) OF SEROPOSITIVE PATIENTS	TOTAL NO. OF CONTROLS	NO. (%) OF SEROPOSITIVE CONTROLS	ADJUSTED ODDS RATIO (95% CI)†
2 mo–4 yr	49	10 (20)	269	16 (6)	4.0 (1.6–10.0)
5–14 yr	124	15 (12)	671	45 (7)	2.2 (1.2–4.2)
≥15 yr	45	6 (13)	225	15 (7)	2.6 (0.9–7.9)
Total	218	31 (14)	1165	76 (7)	2.6 (1.7–4.2)

*Included are codes 141, 143, 144, 145, 147, 148, and 161 of the *International Classification of Diseases, Seventh Revision*.¹⁵

†The odds ratios were adjusted for two levels of cotinine (nonsmoker, <20.00 ng per milliliter; smoker, ≥20.00 ng per milliliter). CI denotes confidence interval.

oral, pharyngeal, and laryngeal cancers.^{35,36} We were not able to control for this possible confounder, but adjusting for the serum cotinine level, a biologic marker of smoking, indicated no confounding by smoking. Smoking is an independent risk factor for head and neck cancer. Two previous reports found no correla-

tion between alcohol consumption and the presence or absence of HPV DNA as detected by PCR in head and neck squamous-cell carcinomas.^{37,38} Our finding that an excess risk was associated with the major oncogenic HPV type (HPV-16), but not with any of the other HPV types that are similarly transmitted, suggests that the HPV-associated risk is not confounded by differences in lifestyle. Our inability to control for risk factors other than smoking in the present study is, however, an important limitation, and the possibility of confounding cannot be disregarded.

A causative association between HPV-16 infection and cancers arising from mucosal squamous-cell epithelium is biologically plausible. HPV-16 can immortalize both cervical and oral epithelial cells *in vitro*.^{39,40} The viral oncoproteins E6 and E7 bind to and inactivate the tumor suppressor proteins p53 and pRb.^{41,42} Identification of HPV (mainly HPV-16) DNA in 11 of 12 tonsillar carcinomas that lacked pRb activity, but in none of 9 tonsillar carcinomas with biologically active pRb, supports the idea that HPV-16 may function in oral carcinogenesis through E7-mediated inactivation of pRb.³¹

Our study does not demonstrate a cause-and-effect relation between HPV-16 infection and squamous-cell carcinoma of the head and neck. Nevertheless, the fact that an excess risk was detectable several years before the diagnosis of cancer indicates that our findings probably cannot be explained by reactivation of virus or an improved ability to detect virus because of the development of cancer.

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