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HHS Public Access

Author manuscript

Cancer Epidemiol Biomarkers Prev. Author manuscript; available in PMC 2015 October 01.

Published in final edited form as:

Cancer Epidemiol Biomarkers Prev. 2015 April ; 24(4): 683–689. doi:10.1158/1055-9965.EPI-14-1217.

Human Papillomavirus 16 E6 Antibodies in Individuals Without Diagnosed Cancer: A Pooled Analysis

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Conflict of Interest: Xavier Castellsagué has had relationships with the following companies: SPMSD, Merck, Genticel.

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Abstract

Background—The increasing incidence of oropharyngeal cancer in many developed countries has been attributed to human papillomavirus type 16 (HPV16) infections. Recently, HPV16 E6 serology has been identified as a promising early marker for oropharyngeal cancer. Therefore, characterization of HPV16 E6 seropositivity among individuals without cancer is warranted.

Methods—4,666 controls were pooled from several studies of cancer and HPV seropositivity, all tested within the same laboratory. HPV16 E6 seropositive controls were classified as having i) moderate (mean fluorescent intensity [MFI]≥484 & <1000) or ii) high seroreactivity (MFI≥1000). Associations of moderate and high HPV16 E6 seroreactivity with i) demographic risk factors; and seropositivity for ii) other HPV16 proteins (E1, E2, E4, E7 and L1) and iii) E6 proteins from non-HPV16 types (HPV6, 11, 18, 31, 33, 45 and 52) were evaluated.

Results—Thirty-two (0.7%) HPV16 E6 seropositive controls were identified; 17 (0.4%) with moderate and 15 (0.3%) with high seroreactivity. High HPV16 E6 seroreactivity was associated with former smoking (odds ratio [OR] 5.5 [95% confidence interval [CI]:1.2-51.8]), and seropositivity against HPV16 L1 (OR 4.8, 95%CI:1.3-15.4); E2 (OR 7.7, 95%CI:1.4-29.1); multiple HPV16 proteins (OR 25.3, 95%CI:2.6-119.6 for 3 HPV16 proteins beside E6) and HPV33 E6 (OR 17.7, 95%CI:1.9-81.8). No associations were observed with moderate HPV16 E6 seroreactivity.

Conclusions—High HPV16 E6 seroreactivity is rare among individuals without diagnosed cancer and was not explained by demographic factors.

Impact—Some HPV16 E6 seropositive individuals without diagnosed HPV-driven cancer, especially those with seropositivity against other HPV16 proteins, may harbor a biologically relevant HPV16 infection.

Keywords

human papillomavirus; HPV16 E6 antibodies; EPIC; ARCAGE; PLCO

Introduction

A rapid increase in the incidence of oropharyngeal cancer has been reported in many parts of the world with a high development index (1-8), especially among men younger than 60 years of age (9). This upsurge has been attributed to an increase in HPV-driven oropharyngeal cancers (7). In the US, incidence has increased by more than 200% over the

past several decades (10). HPV16 infection alone accounts for approximately 90% of HPV-positive oropharyngeal cancers (11, 12) and is estimated to be responsible for at least 50% of oropharyngeal cancer cases in parts of the world with a high development index (10, 13, 14).

Unlike cervical cancer, a precursor lesion for oropharyngeal cancer has yet to be identified, making early detection of oropharyngeal cancers difficult (15). However, numerous case-control studies have shown that the presence of circulating HPV antibodies is strongly associated with cancer of the oropharynx (12, 16-24). Recently, HPV16 E6 antibody positivity has been identified as a potentially promising marker for oropharyngeal cancer (25). A prospective study conducted with prediagnostic sera found that 35% of patients with oropharyngeal cancer were seropositive for HPV16 E6 compared to only 0.6% of controls; for some of the patients these antibodies were present more than 10 years prior to diagnosis and were not associated with cancers at other head and neck cancer sites (25). The specificity of HPV16 E6 marker for detection of oropharyngeal cancer makes biological sense considering that the oropharynx (unlike other anatomic sites of the head and neck) is rich in lymphoid tissue and therefore is more likely to induce an antibody response to HPV infection.

Due to the high specificity of HPV16 E6 seropositivity for oropharyngeal cancer, this marker has the potential to be further developed into a screening tool for identifying high-risk individuals. Therefore, characterization of HPV16 E6 seropositivity within healthy individuals without diagnosed cancer is merited. However, HPV16 E6 seropositivity is extremely rare among healthy individuals without cancer (<1%), making it difficult to study (23-25).

To overcome this issue, we conducted a descriptive epidemiological analysis of pooled controls from several studies of cancer and HPV seropositivity whose samples were all tested within the same laboratory with a bridging panel that allowed for interpretation across studies (23-25). The goals of this analysis were to investigate demographic and serologic factors associated with HPV16 E6 seropositivity among individuals without diagnosed cancer.

Materials and Methods

Our analytic population consisted of 4,666 controls pooled from 4 large studies of HPV seropositivity; 3 studies of head and neck cancer and 1 study of anogenital cancers (23-26). Controls were pooled from i) two nested case-control investigations within the European Prospective Investigation Into Cancer and Nutrition (EPIC); one focused on head and neck cancer (n=1,599 controls) and one focused on HPV-driven anogenital cancers (n=718 controls) (25, 26); ii) 1 nested case-control investigation within the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO) (n=924 controls; unpublished data); and iii) 1 case-control study, the Alcohol-Related Cancers and Genetic Susceptibility in Europe (ARCAGE) (n=1,425 controls) (23). Informed consent was obtained from all participants in each study, and each study was approved by their respective institutional ethics review boards.

Description of Study Populations and Participant Selection

EPIC Cohort Study—In brief, 521,330 individuals were recruited to the cohort between 1992 and 2000 from 10 European countries, of whom 385,747 contributed a blood sample (27). Participants completed self-administered questionnaires on lifestyle factors and diet. Two control participants (one in Denmark) were randomly assigned for each patient with cancer (including head and neck and anogenital cancers) from appropriate risk sets consisting of all cohort participants alive and free of cancer (except non-melanoma skin cancer) at the time of diagnosis (and hence, age) of the index case. Additional study specific matching criteria are discussed below.

EPIC Head and Neck Study: Matching criteria were: country, sex, date of blood collection (± 1 month, relaxed to ± 5 months for sets without available controls), and date of birth (±1 year, relaxed to ± 5 years for sets without available participants). The final study included 1,599 controls (25).

EPIC Anogenital Study: Matching criteria included: study center, sex, date of blood collection (± 3 months, relaxed to ± 6 months for sets without available controls), and age at blood collection (± 3 months, relaxed to ± 2 years for sets without available controls), fasting status, and where relevant, menopausal status, and postmenopausal hormone replacement therapy use, and menstrual cycle. The final study included 718 controls (26).

PLCO Cohort Study—PLCO recruited approximately 155,000 55-74 year-olds from the general population during 1993-2001 who had not been diagnosed previously with prostate, lung, colorectal, or ovarian cancer. Blood (screening arm only), demographic and behavioral data were collected (28). Blood samples were obtained at baseline and five subsequent annual visits; the earliest available sample was used for this study. For each case, four controls were randomly chosen from appropriate risk sets consisting of all cohort members alive and free of cancer (except non-melanoma skin cancer) at the time (and hence age) of diagnosis of the index head and neck cancer case. Matching criteria were: year of entry into the study, year the material was collected, study year of cancer diagnosis (for cases; the same year was used for the matched control), birth year, and smoking status (never, former, current) (unpublished data).

ARCAGE Case-Control Study—Briefly, 2,227 control subjects were recruited from 10 European countries during the period from 2002 to 2005 using a standardized protocol in all centers (except France) (29). All subjects underwent personal interviews to record lifestyle exposures; details are described elsewhere (29). Controls were recruited in each center and frequency matched for age, sex, and area of residence to cases with head and neck cancer. ARCAGE centers mainly used hospital controls to facilitate collection of blood samples, with the exception of the UK centers which used population-based controls randomly chosen from the same family medical practice list as the corresponding cases. Hospital controls were selected from the following diagnoses: endocrine and metabolic, skin, subcutaneous tissue and musculoskeletal, circulatory, nervous system diseases, genitourinary, gastro-intestinal, ear, eye and mastoid, plastic surgery cases and trauma patients (23).

Harmonization of Covariates

Given the variation between studies in how the covariates were ascertained, we were unable to create single unified definitions of smoking and alcohol consumption. As a result, individuals were categorized according to study-specific definitions of smoking and alcohol consumption (Supplemental Table 1).

Harmonization of Serologic Test Results

Plasma (EPIC and ARCAGE) and serum (PLCO) samples were sent on dry ice to the German Cancer Research Center (DKFZ, Heidelberg, Germany) and testing was performed using multiplex assays (24, 30-32). Samples were analyzed for HPV16 antibodies to the major capsid protein (L1), the early oncoproteins (E6, E7), and other early proteins (E1, E2, E4) with the exception of PLCO where seroreactivity against HPV16 E4 was not assessed. Additionally, seroreactivity against the E6 protein from the following HPV types was also evaluated; HPV6, HPV11, HPV18, HPV31, HPV33, HPV45 and HPV52 with the exception of the EPIC anogenital study where seroreactivity against HPV52 was not assessed. A bridging panel was included in all studies so that MFI values could be normalized to account for variation between assays. Briefly, this bridging panel consisted of a subset of 188 samples with known seroprevalence for each HPV antigen as defined by standard MFI cutoffs. For each individual analysis, study-specific cut-off values were calculated for each antigen such that the seroprevalence of each HPV antigen within the bridging panel was the same across all studies. If the study-specific cutoff differed from the standard MFI cutoff by more than 10% for a particular antigen, the MFI values for that antigen were normalized by multiplying each value by the quotient of the standard MFI cutoff divided by the study-specific MFI cutoff. This normalization allowed for the same standard MFI cutoff values for seropositivity to be applied across all three studies.

For HPV16 E6, two mutually exclusive categories of HPV16 E6 seroreactivity were created: i) moderate: MFI ≥ 484 and < 1000; and high: MFI ≥ 1,000. Previous work from our group showed that increasing the seropositivity cutoff of HPV16 E6 from 484 to 1000 resulted in an increased specificity for oropharyngeal cancer without an associated decrease in sensitivity (25). For the other HPV16 proteins the MFI cutoffs used to define seropositivity were: L1, 422; E1, 200; E2, 679; E4, 876; E6, 484; E7, 548. For the E6 proteins of non-HPV16 types the MFI cutoffs for seropositivity were: HPV6 E6, 500; HPV11, 260; HPV18, 243; HPV31, 890; HPV33, 253; HPV35, 260; HPV45, 249; HPV52, 271.

Statistical Analyses

Characteristics of the control participants were evaluated overall and by study. The proportion of HPV16 E6 seropositive controls by demographic categories was computed for the pooled studies. Demographic and serologic determinants of moderate and high HPV16 E6 seroreactivity were evaluated through odds ratios (OR) and 95% confidence intervals (CI) calculated in univariate analyses by logistic regression. Demographic variables evaluated included: gender, age, world region, smoking status and alcohol consumption. Serologic variables evaluated included seroreactivity against: i) other HPV16 proteins (L1, E1, E2, E4, E7) and ii) E6 proteins from non-HPV16 types (HPV6, HPV11, HPV18, HPV31, HPV33, HPV45 and HPV52).

Results

Participant Characteristics

A total of 4,666 controls without diagnosed cancer were included in this analysis (Table 1); 1,425 (30.5%) individuals from ARCAGE; 2,317 (49.7%) from EPIC; and 924 (19.8%) from PLCO. The majority of the controls were male (63.7%), 60 years of age or younger (59.6%) and ever alcohol drinkers (80.3%); smoking status appeared evenly distributed among the categories (i.e.: never, former, current). Small differences between studies were noted for gender, age and smoking status (Table 1).

Demographic Determinants of HPV16 E6 Seropositivity

HPV16 E6 seropositivity was rare. Of the 4,666 pooled controls, a total of 32 individuals (0.7%) were seropositive for HPV16 E6. Prevalence of HPV16 E6 seropositivity was similar by study; ARCAGE (0.8%), EPIC (0.6%) and PLCO (0.9%). Of the 32 HPV16 E6 seropositive controls, 17 (0.4%) were classified as having moderate HPV16 E6 seropositivity (MFI ≥ 484 and < 1000) and 15 (0.3%) were classified as having high HPV16 E6 seroreactivity (MFI ≥ 1000) (Table 2).

Age, gender, smoking status and alcohol consumption did not elevate the odds of moderate or high HPV16 E6 seroreactivity. Only former smoking was significantly associated with high HPV16 E6 seroreactivity, OR 5.5 (95% CI: 1.2-51.8). No other significant associations for either moderate or high HPV16 E6 seroreactivity were observed (Table 2).

Serologic Determinants of HPV16 E6 Seropositivity

Seroreactivity against HPV16 proteins, including L1, E1, E2, E4 or E7, was common. Similar seroprevalence for any of these proteins was observed among the HPV16 E6 seronegative and moderately HPV16 E6 seroreactive controls; 27.9% and 29.4%, respectively (Table 3). No significant associations between moderate HPV16 E6 seroreactivity and seroreactivity against any of the other HPV16 proteins either individually or combined was observed.

In contrast, prevalence of seroreactivity against 1 or more HPV16 proteins (L1, E1, E2, E4 or E7) in addition to E6 was greatest among the controls with high HPV16 E6 seroreactivity; 46.7%. Of the 5 HPV16 proteins evaluated, seroreactivity against HPV16 L1 was most common (n=5 out of 15, 33.3%). Controls with high HPV16 E6 seroreactivity were also more likely than HPV16 E6 seronegative controls to be seroreactive against all HPV16 proteins with the exception of E7, however only HPV16 L1 (OR 4.3, 95% CI: 1.1-13.8) and E2 (OR 7.7, 95% CI: 1.4-29.1) reached statistical significance. High HPV16 E6 seroreactivity was also significantly associated with seroreactivity against multiple HPV16 proteins; OR 25.3 (95% CI: 2.6-119.6) for seroreactivity against 3 HPV16 proteins in addition to E6, although in absolute terms, seroreactivity against multiple HPV16 proteins was rare (4 of 4,666; 0.08%).

7.9% of HPV16 E6 seronegative controls were seroreactive against a non-HPV16 E6 protein compared to approximately 20% for both the moderate and high HPV16 E6 seroreactive

controls. Only seroreactivity for HPV33 E6 was significantly associated with high HPV16 E6 seroreactivity; OR 17.7 (95% CI: 1.9-81.8, Table 4).

Discussion

In this large descriptive epidemiological analysis of more than 4,000 pooled controls from several studies of HPV seroreactivity and HPV-associated cancer (23-25), HPV16 E6 seropositive controls were rare (<1%), particularly those with high HPV16 E6 seroreactivity (0.3%). Further, of the determinants of HPV16 seropositivity evaluated, significant associations were observed only among controls with high HPV16 E6 seroreactivity. Of the demographic risk factors assessed, none were predictors of HPV16 E6 seropositivity except for former smoking. Of the serologic determinants assessed, seroreactivity against other HPV16 proteins was common among all controls and was greatest among controls with high HPV16 E6 seroreactivity (47%). A marker of cumulative lifetime HPV16 exposure, HPV16 L1 seropositivity (5 out of 15; 33.3%) was most commonly detected among high HPV16 E6 seroreactive controls compared to the other 4 HPV16 proteins tested. High HPV16 E6 seroreactivity was significantly associated with seroreactivity against HPV16 L1, E2 and multiple HPV16 proteins. Seropositivity for any non-HPV16 E6 proteins was less common; 8% among HPV16 E6 seronegative controls and approximately 20% among controls with both moderate and high HPV16 E6 seroreactivity. Only HPV33 E6 seroreactivity was significantly associated with high HPV16 E6 seroreactivity, however due to its high sequence homology with the E6 protein of HPV16, this finding may be the result of antibody cross-reactivity (33).

Based on studies of cervical cancer (34), individuals without an underlying HPV-driven cancer would not be expected to have antibodies against the HPV16 oncoproteins. One potential explanation for the small percentage of controls with seroreactivity against HPV16 E6 may be due to potential laboratory error or sample misclassification. Therefore, the 15 strongly HPV16 E6 seroreactive controls identified may reflect the error rates within these large epidemiological studies. Alternatively, the HPV16 E6 seroreactive controls identified in our study may be harboring a yet to be diagnosed cancer or precancer. Recent findings have suggested that induction of HPV-specific antibodies, most notably HPV16 E6 antibodies, may be a response to an underlying HPV-driven neoplastic process that may take years to manifest into a diagnosable cancer (25). Studies conducted at the time of diagnosis have shown that the presence of detectable HPV16-specific antibodies in diagnostic serum is highly sensitive for HPV-driven head and neck squamous cell carcinomas (35). A large proportion (7 out of 15) of controls with high HPV16 E6 seroreactivity was also seroreactive against at least one other HPV16 protein in addition to HPV16 E6. However, of all HPV16 proteins, a recent prospective study showed that seroreactivity against HPV16 E6 is the most strongly associated with development of oropharyngeal cancer; OR 274 (95% CI: 110-681). Of note, all the HPV16 E6 seropositive oropharyngeal cancer cases identified in the previous study had HPV16 E6 MFI values greater than 1,000 and therefore would have been classified as having high seroreactivity in this current analysis (25). Taken together, these findings raise the possibility that the HPV16 E6 seropositive controls with high E6 seroreactivity described in this study, especially those with antibodies against multiple HPV16 proteins, may be on the path to developing an HPV-driven cancer or precancer that,

as follow-up accrues may eventually be diagnosed. Updated record linkage of the EPIC head and neck cancer study (25) revealed that one HPV16 E6 seropositive control was subsequently diagnosed with invasive anal cancer, however, additional follow-up time is needed to fully investigate this possibility.

Therefore, an important limitation of this study is that we are unable to extend follow-up to further ascertain the health status of the HPV16 E6 seropositive controls. For the cohort studies (EPIC and PLCO), we will continue to monitor record linkage updates and investigate this question accordingly; for the ARCAGE case-control studies, no additional follow-up will become available. Additionally, controls in this analysis were initially matched to cases, therefore skewing their distribution of certain variables, such as age and gender, towards that of cases. For the case-control studies, controls were recruited only when they were eligible based on a list of non-chronic diseases unrelated to smoking and alcohol. While this does not jeopardize the validity of our findings, it limits their generalizability. There may have also been some minor misclassification in terms of smoking status and alcohol consumption due to the differences between studies in how these variables were ascertained on the questionnaires. Additionally, we do not have information regarding other covariates such as sexual behavior, and host immunogenetics, which may have been potentially helpful in explaining why some individuals develop these antibodies. Finally, even with over 4,000 pooled controls, we were still limited by power given that our outcome was so rare.

In conclusion, given the rarity of HPV16 E6 seropositivity among individuals without diagnosed cancer, our data suggests that HPV16 E6 serology results in an extremely low rate of misclassification among controls potentially implying that serology may have a higher specificity than other biomarkers in oropharyngeal cancer cases as well. Due to the high specificity of the HPV16 E6 marker (25), HPV16 E6 antibody testing may have the potential to identify individuals at high-risk for developing HPV-driven oropharyngeal cancer within a general population of older adults.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We would like to thank the following people for their contributions to the manuscript: David Castenson (Information Management Services, Calverton MD, USA); Dana Mates (Institute of Public Health, Bucharest, Romania); Vladimir Bencko (Charles University in Prague, Czech Republic); Victor Wünsch-Filho (School of Public Health, University of Sao Paulo, Sao Paulo, Brazil); Elena Matos (Institute of Oncology Angel H. Roffo, University of Buenos Aires, Buenos Aires, Argentina); and Jose Eluf-Neto (Universidade de São Paulo, Sao Paulo, Brazil). We acknowledge contribution of Professor Gary J Macfarlane, Dr Ann-Marie Biggs, Professor Martin Tickle, Professor Phil Sloan and Professor Nalin Thakker with study conduct in UK centers.

Funding Information: This work was supported by the Intramural Research Program of the National Cancer Institute (A.R. Kreimer).

The EPIC study has been supported by the Europe Against Cancer Program of the European Commission (SANCO); Deutsche Krebshilfe, Deutsches Krebsforschungszentrum, German Federal Ministry of Education and Research; Danish Cancer Society; Health Research Fund (FIS) of the Spanish Ministry of Health, Spanish Regional Governments of Andalucia, Asturias, Basque Country, Murcia and Navarra; Catalan Institute of Oncology, Spain;

the ISCIII of the Spanish Ministry of Health (RETICC DR06/0020); Cancer Research UK; Medical Research Council, United Kingdom; Greek Ministry of Health; Stavros Niarchos Foundation; Hellenic Health Foundation; Italian Association for Research on Cancer (AIRC); Italian National Research Council, Fondazione-Istituto Banco Napoli, Italy; Associazione Italiana per la Ricerca sul Cancro-AIRC-Milan; Compagnia di San Paolo; Dutch Ministry of Public Health, Welfare and Sports; World Cancer Research Fund; Swedish Cancer Society; Swedish Scientific Council; Regional Government of Västerbotten, Sweden; NordForsk (Centre of excellence programme HELGA), Norway; French League against Cancer (LNCC), France; National Institute for Health and Medical Research (INSERM), France; Mutuelle Générale de l'Education Nationale (MGEN), France; 3M Co, France; Gustave Roussy Institute (IGR), France; and General Councils of France (P. Brennan).

The ARCAGE study was supported by the grant from European Commission's 5th Framework Program (contract QLK1-2001-00182). This project was partly funded by the Health General Directorate of the French Social Affairs and Health Ministry. The serology testing was supported in part by a grant from the European Commission's 7th Framework Program (contract FP7-HEALTH-2011–282562) (P. Brennan).

PLCO was supported by the Intramural Research Program of the Division of Cancer Epidemiology and Genetics and by contracts from the Division of Cancer Prevention, National Cancer Institute, NIH, DHHS.

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Table 1

Participant characteristics overall and by study

Characteristics	All Studies N= 4666		ARCAGE N= 1425		EPIC N= 2317		PLCO N= 924	
	N	%	N	%	N	%	N	%
Gender								
Male	2972	63.7%	1059	74.3%	1165	50.3%	748	81.0%
Female	1694	36.3%	366	25.7%	1152	49.7%	176	19.0%
Age								
≤60	2782	59.6%	742	52.1%	1726	74.5%	314	34.0%
61-70	1436	30.8%	417	29.3%	504	21.8%	515	55.7%
>70	448	9.6%	266	18.7%	87	3.8%	95	10.3%
Region of Origin[1]								
Eastern Europe	185	4.0%	185	13.0%	0	0.0%	0	0.0%
Northern Europe	1430	30.6%	296	20.8%	1134	48.9%	0	0.0%
Southern Europe	1324	28.4%	757	53.1%	567	24.5%	0	0.0%
Western Europe	803	17.2%	187	13.1%	616	26.6%	0	0.0%
United States	924	19.8%	0	0.0%	0	0.0%	924	100.0%
Smoking[2]								
Never	1770	37.9%	516	36.2%	1040	44.9%	214	23.2%
Former	1614	34.6%	506	35.5%	718	31.0%	390	42.2%
Current	1242	26.6%	403	28.3%	519	22.4%	320	34.6%
Alcohol Consumption[2]								
Never	475	10.2%	172	12.1%	148	6.4%	155	16.8%
Ever	3745	80.3%	1252	87.9%	1782	76.9%	711	76.9%

Abbreviations: European Prospective Investigation Into Cancer and Nutrition (EPIC); Alcohol-Related Cancers and Genetic Susceptibility in Europe (ARCAGE); Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO); mean fluorescence intensity (MFI)

[1] Northern Europe includes: Denmark, Great Britain, Ireland, Norway and Sweden
Southern Europe includes: Croatia, Greece, Italy and Spain
Western Europe includes: France, Germany and The Netherlands
Eastern Europe includes: Czech Republic/Slovakia

[2]Columns do not add to total due to missing data

Table 2

Univariate Analysis of Demographic Characteristics and HPV16 E6 Seropositivity Among Controls

		HPV16 E6 Seropositive			
	Total	Moderate		High	
Characteristics	**N= 4666**	**N= 17**	**OR (95% CI)**	**N= 15**	**OR (95% CI)**
	N	**N (%)**		**N (%)**	
Study					
All	4666	17 (0.4)	-	15 (0.3)	-
ARCAGE	1425	5 (0.4)	-	6 (0.4)	-
EPIC	2317	9 (0.4)	-	4 (0.2)	-
PLCO	924	3 (0.3)	-	5 (0.5)	-
Gender					
Male	2972	11 (0.4)	Ref	10 (0.3)	Ref
Female	1694	6 (0.4)	1.0 (0.3-2.8)	5 (0.3)	0.9 (0.2-2.8)
Age					
≤60	2782	9 (0.3)	Ref	9 (0.3)	Ref
61-70	1436	6 (0.4)	1.3 (0.4-4.1)	5 (0.3)	1.1 (0.3-3.6)
>70	448	2 (0.4)	1.4 (0.1-6.7)	1 (0.2)	0.7 (0.0-5.0)
Region of Origin[1]					
Northern Europe	1430	3 (0.2)	Ref	4 (0.3)	Ref
Southern Europe	1324	6 (0.5)	2.2 (0.5-13.4)	3 (0.2)	0.8 (0.1-4.8)
Eastern Europe	185	1 (0.5)	2.6 (0.0-32.3)	0 (0.0)	-
Western Europe	803	4 (0.5)	2.4 (0.4-16.3)	3 (0.4)	1.3 (0.2-7.9)
United States	924	3 (0.3)	1.6 (0.2-11.6)	5 (0.5)	1.9 (0.4-9.8)
Smoking[2]					
Never	1770	9 (0.5)	Ref	2 (0.1)	Ref
Former	1614	4 (0.2)	0.5 (0.1-1.8)	10 (0.6)	5.5 (1.2-51.8)
Current	1242	4 (0.3)	0.6 (0.1-2.3)	3 (0.2)	2.1 (0.2-25.7)
Alcohol Consumption[2]					
Never	475	2 (0.4)	1.0 (0.1-4.5)	0 (0.0)	N/E

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		HPV16 E6 Seropositive			
	Total	Moderate		High	
Characteristics	N= 4666	N= 17	OR (95% CI)	N= 15	OR (95% CI)
	N	N (%)		N (%)	
Ever	3745	15 (0.4)	Ref	12 (0.3)	Ref

Abbreviations: European Prospective Investigation Into Cancer and Nutrition (EPIC); Alcohol-Related Cancers and Genetic Susceptibility in Europe (ARCAGE); Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO); Not Estimateable (N/E)

[1] Northern Europe includes: Denmark, Great Britain, Ireland, Norway and Sweden
Southern Europe includes: Croatia, Greece, Italy and Spain
Western Europe include: France, Germany and The Netherlands
Eastern Europe includes: Czech Republic/Slovakia

[2] Columns do not add to total due to missing data

Table 3

Association of HPV16 L1, E1, E2, E4 and E7 Seropositivity with Moderate and High HPV16 E6 Seroreactivity Among Controls

	HPV16 E6 Seronegative			HPV16 E6 Seropositive					
				Moderate			High		
HPV16 Proteins	Total	No. Positive (%)	OR	Total	No. Positive (%)	OR (95%CI)	Total	No. Positive (%)	OR (95%CI)
Any[1]	4634	1245 (27.9)	Ref	17	5 (29.4)	1.1 (0.3-3.5)	15	7 (46.7)	2.4 (0.7-7.5)
HPV16 L1	4634	484 (10.4)	Ref	17	1 (5.9)	0.5 (0.0-3.5)	15	5 (33.3)	4.3 (1.1-13.8)
HPV16 E1	4632	155 (3.3)	Ref	17	0 (0.0)	-	15	1 (6.7)	2.1 (0.0-13.7)
HPV16 E2	4634	145 (3.1)	Ref	17	1 (5.9)	1.9 (0.0-12.6)	15	3 (20.0)	7.7 (1.4-29.1)
HPV16 E4[2]	3718	367 (9.9)	Ref	14	3 (21.4)	2.5 (0.4-9.5)	10	2 (20.0)	2.3 (0.2-11.5)
HPV16 E7	4634	345 (7.4)	Ref	17	1 (5.9)	0.8 (0.0-5.0)	15	1 (6.7)	0.9 (0.0-5.9)
Positive for any 2[1]	4634	221 (4.8)	Ref	17	1 (5.9)	1.2 (0.0-8.1)	15	2 (13.3)	3.1 (0.3-13.7)
Positive for any 3[1]	4634	28 (0.6)	Ref	17	0 (0.0)	-	15	2 (13.3)	25.3 (2.6-119.6)
Positive for any 4[1]	4634	2 (0.0)	Ref	17	0 (0.0)	-	15	0 (0.0)	-
Positive for any 5[1]	4634	0 (0.0)	Ref	17	0 (0.0)	-	15	0 (0.0)	-

[1] In addition to HPV16 E6

[2] Seroreactivity against HPV16 E4 was not assessed in PLCO

Table 4

Association of non-HPV16 Type E6 Seropositivity with Moderate and High HPV16 E6 Seroreactivity Among Controls

	HPV16 E6 Seronegative			HPV16 E6 Seropositive					
				Moderate			High		
HPV16 Proteins	Total	No. Positive (%)	OR	Total	No. Positive (%)	OR (95%CI)	Total	No. Positive (%)	OR (95%CI)
Any	4634	368 (7.9)	Ref	17	4 (23.5)	3.6 (0.8-11.6)	15	3 (20.0)	2.9 (0.5-10.8)
HPV6	4634	26 (0.6)	Ref	17	1 (5.9)	11.1 (0.3-76.6)	15	0	-
HPV11	4634	90 (1.9)	Ref	17	0	-	15	1 (6.7)	3.6 (0.1-24.2)
HPV18	4634	56 (1.2)	Ref	17	1 (5.9)	5.1 (0.1-34.0)	15	0	-
HPV31	4634	85 (1.8)	Ref	17	1 (5.9)	3.3 (0.1-22.0)	15	0	-
HPV33	4634	40 (0.9)	Ref	17	0	-	15	2 (13.3)	17.7 (1.9-81.8)
HPV45	4634	65 (1.4)	Ref	17	0	-	15	0	-
HPV52[1]	3920	48 (1.2)	Ref	14	1 (7.1)	6.2 (0.1-42.9)	14	0	-

[1] Seroreactivity against the E6 protein of HPV52 was not determined in the EPIC anogenital study