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Prior Human Polyomavirus and Papillomavirus Infection and Incident Lung Cancer: A Nested Case-Control Study

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Abstract

Purpose—To test whether infection with select human polyomaviruses (HPyV) and human papillomaviruses (HPV) is associated with incident lung cancer.

Methods—We performed a nested case-control study, testing serum from the Carotene and Retinol Efficacy Trial (CARET), conducted 1985–2005, for antibodies to Merkel cell (MCV), KI (KIV), and WU (WUV) HPyVs as well as to six high-risk and two low-risk HPV types. Incident lung cancer cases (n=200) were frequency-matched with controls (n=200) on age, enrollment and blood draw dates, intervention arm assignment, and the number of serum freeze / thaw cycles.

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Compliance with Ethical Standards

Disclosure of potential conflicts of interest: Lisa E. Manhart has received test kits and reagents from Hologic/Gen-Probe over the past 5 years. Jennifer S. Smith has received unrestricted educational grants, consultancy, and research grants from GlaxoSmithKline and Merck Corporation over the past 5 years. You-Lin Qiao has received unrestricted educational grants, consultancy, and research grants from Qiagen, GlaxoSmithKline and Merck Corporation over the past 5 years. All other authors report no potential conflicts.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: Informed consent was obtained from all individual participants included in the study.

Sera were tested using multiplex liquid bead microarray antibody assays. We used logistic regression to assess the association between HPyV and HPV antibodies and lung cancer.

Results—There was no evidence of a positive association between levels of MCV, KIV, or WUV antibodies and incident lung cancer (*P*-corrected>0.10 for all trend tests; odds ratio (OR) range 0.72 to 1.09, *P*-corrected>0.10 for all). There was also no evidence for a positive association between HPV 16 or 18 infection and incident lung cancer (*P*-corrected≥0.10 for all trend tests; OR range 0.25 to 2.54, *P*>0.05 for all OR>1), but the number of persons with serologic evidence of these infections was small.

Conclusions—Prior infection with any of several types of HPyV or HPV was not associated with subsequent diagnosis of lung cancer. Infection with these viruses likely does not influence a person's risk of lung cancer in Western smoking populations.

Keywords

lung cancer; KI polyomavirus; WU polyomavirus; Merkel cell polyomavirus; HPV

Introduction

Carcinoma of the lung is the most commonly diagnosed cancer worldwide and the only cancer among the top ten leading causes of death globally [1]. Seven viruses are strongly associated with the incidence of human cancers [2] and most of these cancers occur at increased rates in immune deficient populations [3]. The lung's propensity for infection and increased lung cancer rates in immunocompromised patients [4] suggest that viral infections may contribute to lung cancer risk.

Merkel cell (MCV), KI (KIV), and WU (WUV) polyomaviruses have been examined in association with lung cancer due to their membership in a carcinogenic viral family [5, 6] and their ability to infect the lower respiratory tract [7–9]. The existing literature is informative, and yet limited in important ways. Prior DNA-based studies of MCV have reported prevalences of 39% (7/18)[10], 16.7% (5/30) [11], 4.7% (4/86) [12], and 17.9% (20/112) [13]. The evidence for an association between KIV or WUV and lung cancer is not consistent. Though an Italian study reported finding KIV DNA in 45% (9/20) of lung tumors compared to 5% (1/20) of adjacent normal tissues [14], a 2009 study of 32 pulmonary tumors and a 2011 study of 30 adenocarcinoma specimens both reported finding no KIV or WUV DNA [15, 16]. To our knowledge, there have been no seroepidemiologic studies of the association between polyomavirus infection and lung cancer. This is a limitation because, unlike nucleic acid amplification test (NAAT) based studies, prospective seroepidemiologic studies may be able to detect the association of viruses that initiate carcinogenesis, but whose viral DNA is no longer detectable in the tumors. Such "hit-and-run" mechanisms have been proposed for the role of MCV in the development some Merkel Cell carcinomas [17] and may be present in other human polyomavirus (HPyV) associated cancers.

Human papillomavirus (HPV) infections are associated with up to 35% of oropharyngeal cancers [18]. In addition, HPV 6 and 11 are involved in the formation of respiratory

papillomas [19], with occasional malignant transformation of infected cells [20]. Based on this carcinogenic potential in the respiratory tract, previous studies have sought to evaluate the association between lung cancer and HPV infection. A 2009 meta-analysis [21] and systematic review [22] evaluated the accumulated evidence and independently concluded that HPV may be a risk factor for some histologies of lung cancer. However, both manuscripts noted the substantial heterogeneity in the reported data and argued that further studies were needed. A 2015 meta-analysis reported an association between HPV infection and lung cancer [23], but others have raised concerns regarding the limited number of included studies and possible confounding [24].

Regardless, the vast majority of previous studies of the association of these viruses with lung cancer utilized cross-sectional designs, and so were unable to establish the temporal sequence of viral infection and incident cancer. We therefore conducted a nested case-control study of lung cancer within a longitudinal study to assess antibodies to MCV, KIV, WUV, and eight HPV types, using liquid bead microarray antibody (LBMA) assays.

Materials and Methods

Study population

The Carotene and Retinol Efficacy Trial (CARET) was a randomized double-blind, multicenter chemoprevention trial, which tested whether supplementation with retinyl palmitate (25,000 ID/day) in combination with β-carotene (30 mg/day) could reduce lung cancer incidence among asbestos exposed participants (n=4,060) and smokers with 20 or more pack-years of exposure (n=14,254) [25]. Participants were recruited from study centers in Seattle, Washington; Portland, Oregon; Irvine, California; San Francisco, California; Baltimore, Maryland; and Groton, Connecticut. The last three locations primarily enrolled asbestos exposed participants. The trial began in May 1985 and was stopped on January 18, 1996 due to evidence of increased risk among those receiving supplementation. Follow-up activities continued through June 30, 2005. The study identified 1,445 incident lung cancer cases during follow-up.

Details of the CARET study have been previously described [26]. Briefly, original CARET staff collected health histories, demographic data, and anthropomorphic measurements. Through 1996, annual in person and semi-annual telephone-based interviews elicited information on relevant signs, symptoms, and new medical diagnoses. Participants also received brief physical exams during the yearly study center visit. Participants provided baseline (pre-randomization) serum, and additional sera samples were collected at 2-year intervals thereafter. From 1996 through 2000 contact was limited to one phone call per year and from 2000–2005 questionnaires were the primary point of contact, with phone calls given to follow-up with non-responders. All sera were stored at −70°C in the biorepository of the CARET Coordinating Center at the Fred Hutchinson Cancer Research Center in Seattle, WA. We excluded all asbestos exposed participants from this analysis.

All participants provided signed informed consent, and the institutional review boards at each trial center reviewed and approved CARET activities annually [25]. The institutional

review boards of the University of Washington and the Fred Hutchinson Cancer Research Center approved this current analysis.

Case definition

Through February 28, 1998, the CARET end-points review committee obtained clinical records and requested pathology or cytology specimens for independent review by the CARET pathologist. Three independent physician adjudicators determined the origin, location, histology and date of lung cancer diagnosis [27]. Beginning March 1, 1998, pathology reports from diagnosing institutions, without independent specimen review by the CARET pathologist, were reviewed by the adjudicators [27]. After October 1, 1998, CARET endpoint specialists reviewed the pathology reports, with adjudication by a single independent physician [27]. Searches of local cancer registries and the National Death Index were used to identify cases among those lost to follow-up. Self-report was not considered adequate evidence of case status for those included in our study.

Cases were defined as individuals with incident lung cancer of any histology (small cell carcinoma (SCLC), adenocarcinoma (ADC), squamous cell carcinoma (SCC), large cell carcinoma (LCLC), non-small cell lung cancer not otherwise specified (NSCLC, NOS), and unknown). We randomly selected a sample of these cases (n=200) from participants who had been free of all cancers prior to lung cancer diagnosis and who had serum available (with no more than two freeze-thaw cycles) from a blood draw that took place 366 to 1095 days prior to diagnosis.

We selected control specimens (n=200), using incidence density sampling with replacement, among participants who were cancer free and had available serum with no more than two freeze-thaw cycles. All controls were alive at the time that the cases were diagnosed with lung cancer. There were eight instances where an individual contributed sera a second time. Controls were frequency matched on age at enrollment (five-year age groups), intervention arm assignment (supplementation with retinyl palmitate in combination with β-carotene vs. placebo), year of enrollment, date of blood draw (six month intervals), and the number of serum freeze / thaw cycles.

Exposure measurement

We performed a liquid bead microarray antibody assay following an established protocol [28, 29] with previously described modifications [6]. We used a Bio-Plex 200 instrument (Bio-rad Laboratories, Hercules, CA) to obtain the median fluorescence intensity (MFI), a measure of the strength of antibody response. We tested sera for antibodies against the primary structural protein (VP1) and the small T antigen (ST-Ag), an oncoprotein, of MCV, KIV, and WUV. We also tested for antibodies against the major structural protein (L1) of six high-risk (16, 18, 31, 33, 52, and 58) and two low-risk (6 and 11) HPV types. In addition, we tested for antibodies to the E6 and E7 oncoproteins of HPV types 16 and 18. Because of the expected high prevalence of BK seropositivity [30], antibodies to BK polyomavirus VP1 antigens served as a positive control and glutathione S-transferase (GST)/ “Tag” [31] fusion proteins were used to determine the background MFI [32]. According to previously described criteria (MFI>400) [30], 91.3% of our sera were

seropositive for BK VP1. Existing fusion proteins were used for all antigens except for KIV ST-Ag (NCBI Reference Sequence: NC_009238.1) and WUV ST-Ag (NCBI Reference Sequence: NC_009539.1). We designed novel fusion proteins with the "Tag" 11-amino acid sequence on the C-terminus and expressed them in pEX-N-GST vectors (Blue Heron Bio, Bothell, WA) so that GST was fused to the N-terminus.

Quality control

Each 96-well plate included four (two sets of two) quality control specimens randomly distributed among the utilized wells. We assessed the reproducibility of the results by examining the distribution of quality control sample MFI values across plates. For example, for the first quality control set, the lnMFI for HPV 6 L1 ranged from 5.06 to 7.08, with a standard deviation (SD) of 0.43 and the lnMFI for MCC VP1 ranged from 8.87 to 9.89, with a SD of 0.30. For the second quality control set, the parallel ranges and SD were 5.97 to 7.86 (SD=0.49) and 8.08–9.84 (SD=0.30). In addition, an equal number of case and control specimens were assigned to each plate to ensure that any plate to plate variation would be equally distributed among cases and controls. Laboratory personnel were blinded with regard to which specimens were cases, controls, or quality control samples.

Statistical methods

Power calculations for all antibodies accounted for log MFI as a continuous predictor, and were performed by reducing the calculations to a comparison of the means of two normally distributed variables using a two-sample T-test [33]. Assuming a sample size of 400, 5% false positive proportion (α=0.05), a 1:1 ratio of cases and controls, equal variances between cases and controls, and a standard deviation of 0.8, the minimum detectable difference in log MFI at 80% power was 0.23 and at 90% power it was 0.26.

Because prior infection with polyomaviruses is nearly ubiquitous [34], and previous findings that high levels of MCV MFI were associated with Merkel cell carcinoma despite MCV infection being common [6], we evaluated quartiles of HPyV MFI levels. The lowest quartile served as the referent in logistic regression analyses. In order to maximize our study's comparability with previous studies of HPV using LBMA, we defined HPV seropositivity as >400 MFI in our primary logistic regression analysis [28, 35, 36], and as >200 MFI in a sensitivity analysis [36, 37]. We also performed logistic regression linear trend tests for the association between lung cancer and the MFI for each viral antibody. We grouped HPyV analyses into three exposure categories: MCV (VP1 & ST-Ag), KIV (VP1 & ST-Ag), and WUV (VP1 & ST-Ag). HPV infection was grouped into four categories: HPV-16 (E6, E7, and L1), HPV-18 (E6, E7, and L1), other high-risk HPV (31, 33, 52, 58 L1), and low-risk HPV (6, 11 L1). MFI were natural log transformed (lnMFI) to improve normality and we adjusted all logistic regression analyses for matching variables. As an exploratory exercise, we used boxplots to assess the association of individual histology types (SCLC, ADC, SCC, LCLC, NSCLC, NOS, unknown) with antigen-specific MFI.

We used permutation tests with 10,000 permutations to determine the null distribution of the most significant exposure across the multiple exposures and thereby correct our *P*-values for multiple comparisons [38]. Corrected *P*-values were defined as the proportion of the

permuted datasets with a test statistic as or more extreme than the test statistic calculated from the empirical data.

We assessed effect modification by smoking history (pack-years) and sex using likelihood ratio tests. If smoking history and sex were not effect modifiers, they were considered potential confounders, along with family history of lung cancer (yes, no). Confounding by smoking was assessed by modeling pack-years both as a continuous variable and a categorical variable (20–35 pack-years, 35–50 pack-years, 50–65 pack years, and 65+ pack-years). Confounders were retained in the final model only if inclusion in the model changed the odds ratios (OR) of interest by ≥10%. Analyses used two-sided statistical tests and were performed with Stata/IC 13.1 (StataCorp LP, College Station, TX).

Results

Of the 400 blood specimens, 191 were from the Seattle study site, 130 from Portland, and 79 from Irvine, with no evidence of an association between study site and case status (chi-squared p-value=0.429). There were also no significant differences between cases and controls with respect to year of enrollment, year of blood draw, and the number of serum freeze / thaw cycles (data not shown) nor with age at enrollment and intervention arm assignment (Table 1), suggesting frequency matching was successful. The median age at enrollment was 61.6 years (interquartile range 57.2 – 65.0) and 58.5% of participants had been assigned to the intervention arm. The sampled population was approximately 41% female and 95% white. More than 50% of cases and controls had at least a college education and approximately 71% were married. The distribution of BMI was different between cases and controls (P=0.034), with fewer cases (65.3%) being overweight or obese than controls (73.5%). Though not statistically different, 75.5% of cases were current smokers at enrollment compared to 67.0% of controls. Though all participants had at least 20 pack-years of smoking exposure, on average cases had higher-level exposures (P< 0.001). A family history of lung cancer was reported by almost twice as many cases (16.0%) as controls (8.5%) (P=0.032). Asthma was reported by nearly 9% of participants and tuberculosis by 1% and did not differ between cases and controls. Chronic bronchitis or emphysema was reported by 22% of cases and 15.5% of controls (P=0.095). A history of pneumonia was reported by 30% of cases and 21.5% of controls (P=0.051).

The majority (95.5%) of lung cancer cases were confirmed by pathology reports, eight (4%) were clinical diagnoses confirmed by medical records, and one case (0.5%) was based solely on a death certificate. With regard to histology, 22% (n=44) of cases were diagnosed as SCLC, 73% (n=146) as NSCLC, and 5% (n=10) as unknown. Among NSCLC cases, 45% (n=66) were diagnosed as ADC, 25% (n=37) as LCLC, 21% (n=31) as SCC, and 8% (n=12) as NSCLC, NOS (data not shown).

The distributions of case and control lnMFI were similar for all assessed antibodies (P>0.05) (Table 2).

Analysis of HPyV MFI quartiles provided no evidence of a positive association with incident lung cancer (Table 3). With the lowest quartile as the referent, odds ratios ranged

from 0.72 (95% CI: 0.41–1.26, *P*-corrected=0.26) for the highest quartile of MCV ST-Ag to 1.09 (95% CI: 0.62–1.90, *P*-corrected=0.76) for the highest quartile of KIV VP1. HPyV trend tests were not statistically significant and family history of lung cancer, smoking, and sex were neither effect modifiers nor confounders (data not shown).

Based upon a cut-point of 400 MFI, there was no evidence of a positive association between HPV seropositivity and incident lung cancer (Table 4). Odds ratios ranged from 0.25 (95% CI: 0.08–0.77, *P*-corrected=0.01) for HPV 16 L1 to 2.54 (95% CI: 0.49–13.34, *P*-corrected=0.30) for HPV 16 E6. Significant inverse associations were also found between HPV 31 L1 (OR=0.48, 95%: 0.24–0.95) and HPV 33 L1 (OR=0.40, 95% CI: 0.18–0.90) seropositivity and case status. Sensitivity analyses, with a seropositivity cut-point of 200 MFI, also showed no evidence of a positive association (Supplementary Table 1). Trend tests for individual HPV antibodies were not statistically significant (*P*-corrected≥0.10 for all). Family history of lung cancer, smoking, and sex were not effect modifiers or confounders (data not shown).

In exploratory analyses, we found no evidence of an association between specific HPyV or HPV antibodies and any individual histologic type of lung cancer (Supplementary Figures 1a, 1b, and 1c).

Discussion

In this primarily Caucasian population of heavy smokers, we found no evidence of a positive association between HPyV antibodies or HPV seropositivity and incident lung cancer, whether considered as a whole or as individual histologic types.

To our knowledge there have been no previous seroepidemiologic studies of the association between HPyV infection and lung cancer. However, some prior NAAT based studies have reported associations of MCV and KIV DNA with lung tumors. An American study reported a prevalence of 16.7% (5/30) for MCV DNA in NSCLC compared to 9.5% (2/21) in benign adjacent tissue [11], a difference that was not statistically significant (*P*=0.47). In addition, a German study of MCV in SCLC reported that 39% (7/18) of lung tumors had MCV DNA compared with 0% (0/18) of controls [10]. Though this was statistically significant (*P*=0.003), controls were blood samples rather than lung tissue and the smoking history was not assessed. A Chilean study that reported a 4.7% (4/86) prevalence among ADC and SCC lacked controls entirely [12], as did a Japanese study that reported a prevalence of 17.9% (20/112) [13]. Therefore, our null results suggest that MCV DNA may be present in healthy as well as cancerous lung tissue. It is also possible that many of the DNA positive lung specimens represent transient infections, which are unrelated to lung cancer initiation. As in the US study mentioned above, the Italian study that reported a positive association for KIV DNA in lung tumors used surrounding normal tissue as the controls [14]. However, that study was small (n=40) and other studies of KIV and WUV in lung tumors found no evidence of infection with these viruses in lung tumors [15, 16].

In our primary HPV analysis, there were three type-specific antibodies with P-corrected <0.05. In each of these instances the OR was <1, and was therefore counter to our

hypothesis. However, it is possible that higher HPV antibody levels better protect against recurrence or reactivation of HPV infection, thereby hindering the development of HPV-associated cancers. Alternatively, these strong immune responses may be indicative of individuals with highly competent immune systems, which are able to protect against the development or progression of lung cancer through other non-virus related mechanisms.

The seroprevalence of HPV 16 and 18 L1 was lower than expected. However, archived sera known to be seroreactive were also assayed on each plate as controls and consistently produced substantially higher MFI. Furthermore, all of the sera were either strongly reactive to BK (the positive control) or at least one other antigen, therefore the antibodies in the sera were unlikely to have been substantially degraded by freezing and thawing. Based on reports that smokers are less likely to seroconvert when infected with oncogenic HPVs [39], some of these atypical results may be explained by the high levels of smoking in this study population.

The lack of a positive association between HPV seropositivity and incident lung cancer in our data is consistent with the results of several studies: 1) a Finnish nested case-control study of HPV 16 and 18 infections and female lung cancer [40]; 2) the nested case-control portion of a recently published large European study of HPV 6, 11, 16, 18, or 31 antibodies and lung cancer [41]; and 3) robust NAAT-based studies of a variety of HPV types and lung cancer in Western populations [41, 42]. However, other studies report associations between HPV and lung cancer, and there is substantial heterogeneity in the literature. In 2012, a systematic review and meta-analysis reported stronger associations in Asia compared to Australia, Europe, and North America [43]. The 2015 meta-analysis also reported a positive association, but it was based on only nine studies, six of which were in East Asia [23]. While there may be true variation in HPV's association with lung tumors due to variation in sexual practices (e.g., engagement in oral sex [42]) or other exposures, laboratory methods may also contribute to this heterogeneity [21]. For example, both of the large null European NAAT-based studies took extensive precautions to avoid DNA contamination [41, 42].

There are more than a dozen discovered HPyVs, and we chose to assess antibodies to MCV due to its proven carcinogenic potential. We evaluated KIV and WUV due to their discovery in respiratory secretions. Since the host cell tropism of many of the recently discovered HPyVs is uncertain [44], it is possible that other HPyVs may also infect the lungs. Among the remaining HPyVs [44–49], only MX was also initially discovered in respiratory secretions [50]. However, MX was 4.5 times more likely to be found in fecal samples than in respiratory specimens [50], suggesting that is not primarily a respiratory pathogen.

This study has several limitations. First, all participants were current or former heavy smokers, and the carcinogenic effect of this tobacco exposure may have overwhelmed our ability to detect a small absolute increase in risk associated with viral infections. Assessing these associations among lung cancer patients without a smoking history may yield different results. Second, our sample size was limited and did not allow for meaningful stratification by histological subtypes of lung cancer. Third, while we tested for L1 antibodies to all included HPV types, antibodies to viral oncoproteins E6 and E7 were only assessed for HPV 16 and 18. Since L1 antibodies are markers of HPV infection, rather than specific to HPV-

induced cancers, our ability to determine an association with the remaining six HPV types was comparatively weaker. Despite antibody cross-reactivity for E6 and E7 [51], our study lacked power to indirectly detect antibodies to these proteins in non-16 and non-18 HPV types. Fourth, serology may be less sensitive than nucleic acid amplification-based tests. Although there are reports of high concordance of serology with DNA based tests for HPV-positive head and neck tumors [52], we cannot assume the same immunologic response in lung cancer. Fifth, our assay was unable to determine the site of infection. If possible, future similar studies should be conducted using biorepositories with available tumor specimens to confirm positive serologic associations. Sixth, although HPyV analyses were adequately powered, our study lacked sufficient statistical power to examine a potential role of infection with HPV 16 and 18. Finally, we did not exclude cases identified solely on the basis of a death certificate, which could have introduced misclassification of case status compared to those confirmed by pathology reports and other clinical diagnoses. However only one of our cases was diagnosed in this way so any resulting bias would be minimal.

Despite these limitations, assessment of serum antibodies rather than viral nucleic acids was a strength of this study. Compared to NAATs, antibody assays are less susceptible to contamination. This is potentially important since concerns have been raised that some previously reported positive results may have been the result of contamination [21]. In addition, as noted above, antibody based studies could allow for the detection of viral infections that initiated carcinogenesis, but whose viral DNA is no longer present in tumor tissue. Furthermore, assessing antibodies in prospectively collected sera establishes temporality, demonstrating that infection preceded the development of lung cancer, thereby strengthening causal inference. Finally, because obtaining blood samples is minimally invasive, relatively inexpensive, and considered both ethically and socially acceptable, serum based studies allow for the selection of genuinely comparable controls. Because sampling lung tissue is invasive, and therefore unethical, many nucleic acid based studies of viral infections and lung cancer have had no comparison group.

In summary, in this population of heavy smokers from the U.S., there was no evidence of an association between HPyV antibody levels or prior HPV infection and the development of lung cancer. These findings, in conjunction with broadly-similar findings in other studies [41, 42], suggest that neither HPV nor HPyV infections are associated with lung cancer in Western populations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Characteristics of selected incident lung cancer cases and frequency matched controls from the Beta-Carotene and Retinol Efficacy Trial (CARET) for lung cancer chemoprevention.

	Cases (n=200)		Controls (n=200)		P-value
	N	(%)	N	(%)	
Age at enrollment (years)					1.000
50–54	35	(17.5)	34	(17.0)	
55–59	41	(20.5)	42	(21.0)	
60–64	74	(37.0)	75	(37.5)	
65–70	50	(25.0)	49	(24.5)	
Intervention arm[a]	117	(58.5)	117	(58.5)	1.000
Female sex	78	(39.0)	85	(42.5)	0.542
White race	189	(94.5)	191	(95.5)	0.819
Education level[b]					0.119
Grade school	3	(1.8)	6	(3.5)	
High school	65	(38.0)	52	(30.6)	
College	88	(51.5)	85	(50.0)	
Graduate school	15	(8.8)	27	(15.9)	
Married	144	(72.4)	140	(70.4)	0.740
Body Mass Index (BMI) (kg/m^2)					0.034
Underweight (<18.5)	4	(2.0)	1	(0.5)	
Normal (18.5–24.99)	65	(32.7)	52	(26.0)	
Overweight (25–29.99)	93	(46.7)	88	(44.0)	
Obese (≥30)	37	(18.6)	59	(29.5)	
Current smoker	151	(75.5)	134	(67.0)	0.077
Pack-years of smoking					<0.001
20–35	21	(10.5)	38	(19.0)	
35–50	69	(34.5)	91	(45.5)	
50–65	44	(22.0)	37	(18.5)	
65+	66	(33.0)	34	(17.0)	

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	Cases (n=200)		Controls (n=200)		P-value
	N	(%)	N	(%)	
Years since quitting smoking					0.431
<1	4	(8.2)	7	(10.6)	
1–2	24	(49.0)	22	(33.3)	
3–4	12	(24.5)	21	(31.8)	
5–6	9	(18.4)	16	(24.2)	
Family history of lung cancer	32	(16.0)	17	(8.5)	0.032
History of respiratory illness					
Asthma	20	(10.0)	14	(7.0)	0.286
Tuberculosis	2	(1.0)	2	(1.0)	1.000
Chronic bronchitis or emphysema	44	(22.0)	31	(15.5)	0.095
Pneumonia	60	(30.0)	43	(21.5)	0.051

[a] Supplementation with retinyl palmitate in combination with β-carotene vs. placebo

[b] Highest educational level started, missing 15% of the data

Table 2

The distribution of antigen specific antibodies[a] among lung cancer cases and controls.

	Cases (n=200)		Controls (n=200)			
Antibody	**Mean**	**SD**	**Mean**	**SD**	**Difference**	***P***
HPyV						
MCPyV[b] VP1[c]	7.7	2.5	7.9	2.4	−0.2	0.273
MCPyV[b] ST-Ag[d]	2.8	2.2	3.0	2.2	−0.2	0.576
KIV[e] VP1 [c]	8.5	1.3	8.4	1.4	0.1	0.753
KIV[e] ST-Ag[d]	4.4	0.9	4.5	0.8	−0.1	0.251
WUV[f] VP1 [c]	8.8	0.9	8.9	0.7	−0.1	0.636
WUV[f] ST-Ag[d]	2.6	1.2	2.6	1.4	0.0	0.656
HPV[g]16						
E[h]6	1.4	1.9	1.4	1.8	0.0	0.871
E[h]7	2.0	2.5	2.1	2.6	−0.1	0.84
L[i]1	1.0	1.8	1.2	2.2	−0.2	0.193
HPV[g]18						
E[h]6	0.8	1.3	0.9	1.3	−0.1	0.531
E[h]7	0.6	1.1	0.7	1.4	−0.1	0.399
L[i]1	3.9	1.0	4.0	1.0	−0.1	0.169
Other high-risk HPV[g]						
31 L[i]1	2.8	2.1	3.1	2.3	−0.3	0.161
33 L[i]1	2.3	2.2	2.4	2.4	−0.1	0.434
52 L[i]1	4.0	1.0	4.2	1.0	−0.2	0.084
58 L[i]1	1.0	1.5	1.0	1.6	0.0	0.827
Low-risk HPV[g]						
6 L[i]1	5.5	1.5	5.6	1.5	−0.1	0.269

	Cases (n=200)		Controls (n=200)			
Antibody	**Mean**	**SD**	**Mean**	**SD**	**Difference**	***P***
11 L[i]1	3.3	1.8	3.4	2.1	−0.1	0.569

[a]Measured in units of median fluorescence i ntensity (MFI), a m easure of the strength of an antibody response. MFI were natural log transformed to improve normality.

[b]MCPyV =Merkel cell polyomavirus

[c]VP1 = the primary structural protein of human polyomaviruses

[d]ST-Ag = the small T-antigen of human polyomaviruses

[e]KIV = KI polyomavirus

[f]WUV = WU polyomavirus

[g]HPV = Human papillomavirus

[h]E =early protein of human papillomaviruses

[i]L = late protein of human papillomaviruses

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Table 3

Association between antigen specific human polyomavirus (HPyV) antibodies[a] and incident lung cancer, adjusted for matching variables.

Antibody quartile	Mean lnMFI[b]	OR (95%CI[c])	*P*[d]	Trend Test[e]	
				OR (95%CI[c])	*P*[d]
MCPyV[f]VP1[g]				0.96 (0.88–1.04)	0.28
Lowest	4.24	Referent			
Highest	10.06	0.79 (0.45–1.37)	0.40		
MCPyV[f]ST-Ag[h]				0.98 (0.8 9–1.06)	0.58
Lowest	0.04	Referent			
Highest	5.73	0.72 (0.41–1.26)	0.26		
KIV[i]VP1[g]				1.02 (0.89–1.18)	0.76
Lowest	6.66	Referent			
Highest	9.63	1.09 (0.62–1.90)	0.76		
KIV[i]ST-Ag[h]				0.87 (0.69–1.10)	0.25
Lowest	3.33	Referent			
Highest	5.33	0.80 (0.46–1.40)	0.44		
WUV[j]VP1[g]				0.94 (0.74–1.20)	0.65
Lowest	7.78	Referent			
Highest	9.60	0.98 (0.56–1.71)	0.94		
WUV[j]ST-Ag[h]				0.97 (0.8 3–1.12)	0.66
Lowest	0.69	Referent			
Highest	4.01	0.75 (0.43–1.31)	0.32		

[a] Measured in units of median fluorescence intensity (MFI), a measure of th e strength of an antibody response.

[b] lnMFI = natural log transformed MFI

[c] Nominal (uncorrected) 95% confidence intervals

[d] *P*-values are corrected for multiple comparisons using permutation tests.

[e] The trend tests estimate the odds ratio for a one unit increase in natural log transformed MFI, adjusted for matched variables.

[f] MCPyV =Merkel cell polyomavirus

[g] VP1 = the primary structural protein of human polyomaviruses

[h] ST-Ag = the small T-antigen of human polyomaviruses

[i] KIV = KI polyomavirus

[j] WUV = WU polyomavirus

Table 4

Association between human papillomavirus (HPV) seropositivity, defined as >400 MFI[a], and incident lung cancer, adjusted for matching variables.

Antibody	Cases (n=200) %	Controls (n=200) %	OR (95% CI[c])	P[d]	Trend Test[b] OR (95% CI[c])	P[d]
HPV[e]16						
E[f]6	2.5	1.0	2.54 (0.49–13.34)	0.30	1.01 (0.91–1.12)	0.88
E[f]7	8.5	11.0	0.74 (0.38–1.46)	0.44	0.99 (0.92–1.07)	0.85
L[g]1	2.0	7.5	0.25 (0.08–0.77)	0.01	0.94 (0.85–1.03)	0.20
HPV[e]18						
E[f]6	0.5	–	N/ A	-	0.95 (0.82–1.11)	0.54
E[f]7	0.5	0.5	1.00 (0.06–16.72)	1.00	0.94 (0.80–1.09)	0.42
L[g]1	–	1.5	N/A	-	0.87 (0.71–1.06)	0.17
Other high-risk HPV[e]						
31 L[g]1	7.0	13.5	0.48 (0.2 4–0.95)	0.03	0.94 (0.8 6–1.03)	0.17
33 L[g]1	4.5	10.5	0.40 (0.18–0.90)	0.03	0.97 (0.89–1.05)	0.45
52 L[g]1	0.5	1.5	0.33 (0.03–3.18)	0.34	0.83 (0.68–1.03)	0.10
58 L[g]1	0.5	1.0	0.50 (0.04–5.85)	0.57	0.99 (0.87–1.12)	0.84
Low-risk HPV[e]						
6 L[g]1	34.5	40.5	0.77 (0.5 1–1.16)	0.21	0.93 (0.8 1–1.06)	0.27
11 L[g]1	6.5	6.0	1.09 (0.48–2.47)	0.84	0.97 (0.87–1.08)	0.56

[a] MFI = median fluorescence intensity, a measure of the strength of an antibody response.

[b] The trend tests estimate the odds ratio for a one unit increase in natural log transformed MFI, adjusted for matched variables.

[c] Nominal (uncorrected) 95% confidence intervals

[d] *P*-values are corrected for multiple comparisons using permutation tests.

[e] HPV = Human papillomavirus

[f] E =early protein of human papillomaviruses

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g_L = late protein of human papillomaviruses