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Prospective study of JC virus seroreactivity and the development of colorectal cancers and adenomas

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Abstract

Background—Infection with JC virus (JCV) has been proposed as a risk factor for colorectal cancer (CRC). A nested case-control study was conducted to evaluate the association between prediagnostic JCV antibodies and risk of incident CRC and adenomas.

Methods—Two research serum banks were established in Washington County, MD in 1974 and 1989, with the collection of blood samples from >45,000 volunteers. Incident CRC cases diagnosed through 2006 (n=611) were identified among participants by linkage to population-based cancer registries, contributing 729 pairs of observations. Cases of adenomatous polyps (n=123) were identified from participants of the 1989 cohort who reported having a colonoscopy-detected adenoma at follow-up through 2000 with histology confirmed through medical record review. One control was matched to each case on age, sex, race and date of blood draw, and, for adenoma controls, date of endoscopy. IgG antibodies to JCV were measured using virus-like particle ELISA. Associations between JCV seropositivity and CRC and adenomas were estimated using conditional logistic regression.

Results—Overall, there was no association between antibodies to JCV and CRC (odds ratio (OR) =0.91, 95% confidence interval (CI)=0.71–1.17). However, a statistically significant positive association between JCV seropositivity and subsequent adenoma diagnosis was observed among males (OR=2.31, 95% CI=1.20–4.46), while a statistically significant inverse association was observed among females (OR =0.31; 95% CI=0.14–0.67; p for interaction=0.01), after adjustment for baseline smoking and body mass index.

Conclusions—Overall, JCV seropositivity was not associated with CRC development up to 31 years later. Future studies are needed to confirm the adenoma findings.

Keywords

JC virus; colorectal cancer; colorectal adenoma

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INTRODUCTION

Infection with JC virus (JCV) has been proposed as a potential risk factor for cancer, including colon cancer. JCV is a common polyomavirus infection, with antibody seroprevalence estimates ranging from 44% to 75% among U.S. adults [1]. Initial infection with JCV is asymptomatic and usually occurs in later childhood and adolescence, after which the virus remains latent in the kidneys [2]. Reactivation of latent JCV infection can occur under conditions of severe immunosuppression, causing progressive multifocal leukoencephalopathy (PML) in AIDS patients. However, JCV is detected in 37–47% of urine samples from immunocompetent individuals [3–5], suggesting that reactivation of latent infection with active JCV replication is a common phenomenon among healthy adults. The route of JCV person-to-person transmission is unclear. JCV has been detected at high levels from in raw sewage from urban areas throughout the world [6] and in shellfish [7], indicating that humans may be exposed to JCV virus through food and water contaminated by urine and/or feces [7,8]. JCV DNA can remain intact at low pH levels [9], supporting its viability as an infection of the gastrointestinal tract, and JCV viral sequences have been identified from normal human colon mucosa [10].

Several laboratory studies suggest that JCV many play a role in carcinogenesis. JCV encodes a non-structural protein called the large tumor (T) -antigen, which initiates viral DNA replication, stimulates host DNA synthesis, and modulates gene transcription [2]. Large T-antigen has been shown to impair DNA repair processes [11] and can inhibit apoptosis by binding to and inactivating the tumor suppressor proteins p53 and pRb [12]. In addition, recent evidence supports a role for JCV T-antigen in the disruption of the Wnt signaling pathway implicated in colorectal cancer (CRC) carcinogenesis [13–15]; co-expression of β-catenin and JCV T-antigen results in increased transcription of c-myc [14], and JCV-transfected cells exhibit nuclear accumulation of β-catenin and chromosomal aberrations, specifically when JCV T-antigen is expressed [15].

Given the widespread exposure to JCV and the virus's oncogenic potential, several laboratories have investigated the prevalence of JCV in human colorectal cancer (CRC) tissues and adenomas. Seven studies detected JCV DNA sequences in CRC tissues by PCR, ranging in prevalence from 26% to 96% [13,16–21]. Two of the positive studies observed higher viral loads in CRC compared to paired adjacent normal mucosa [18,21], although the absolute viral load in cancer tissues was low (1 copy per 100 cells) [18]. Four studies investigated JCV in adenomas, detecting JCV DNA in 5–82% of samples tested [17,20–22]. JCV T-antigen expression was detected by immunohistochemistry in 77% of JCV DNA-positive CRC tissues [13] and 10–20% of JCV DNA-positive adenomas [20,22]. In contrast to these positive studies, two studies with larger sample sizes (n=100 and 233) did not detect JCV DNA in CRC tissues [23,24]. Reasons for inconsistency across tumor studies may include differences in sample preparation, JCV detection methods and/or the underlying patient populations. Only one study has investigated the association between JCV infection and CRC risk using serology: among 386 CRC cases and 386 matched controls selected from male participants in a prospective cohort in Norway, antibodies against JCV at baseline were not associated with an increased risk of developing colorectal cancer [25].

To further investigate the potential role of JCV in CRC, a prospective study of JCV antibodies and incident CRC and adenomas was conducted in males and females.

METHODS

Study design and population

A nested case-control study was conducted within two community-based cohorts in Washington County, Maryland. The two cohorts were established in 1974 (n=23,951) and 1989

(n=25,080), and named CLUE I and II, respectively, referring to the recruitment campaign slogan, “Give us a clue to heart disease and cancer”. After obtaining written informed consent from all participants, serum (1974) and plasma (1989) were obtained and stored at −70°C. (The term “serum” will be used to describe both types of samples from this point forward.) Participants completed a brief baseline questionnaire at the time of blood donation. Additional follow-up questionnaires were mailed to CLUE II participants in 1996, 1998, 2000 and 2003.

Cases of colorectal cancer (CRC) occurring among CLUE cohort members through July 2006 were identified by linkage to the Washington County Cancer Registry, which has been maintained since 1958, and linkage to the Maryland Cancer Registry since 1992. ICD-9 codes (153 and 154) were used to identify CRC cases diagnosed in 1992–2000, and ICD-10 codes have been in use since 2001 (C18, C19 and C20). Cases were defined as participants of CLUE I or CLUE II who were subsequently diagnosed with CRC, where CRC was their first cancer diagnosis with the possible exceptions of non-melanoma skin cancer (NMSC) or cervical cancer in situ. Cases had to have been Washington County residents at both the time of baseline blood donation and CRC diagnosis. A total of 611 CRC cases were identified among cohort participants, including 118 who participated in both CLUE I and II and contributed blood samples from both timepoints (n=729 blood samples from 611 CRC cases). One control was matched to each CRC case on sex, race, age within one year, cohort (CLUE I, CLUE II, or both), and date of blood draw within two weeks (n=729 blood samples from 611 matched controls, including 118 controls who participated in both CLUE cohorts). Controls for the CRC cases were defined as residents of Washington County at the time of blood donation who were not known to have died or developed cancer (except for possibly NMSC or cervical cancer in situ) as of the date of diagnosis of the case. Vital status is determined through daily searches of obituaries, monthly reviews of county death certificates, annual reviews of state death certificates, and the National Death Index.

Cases of colorectal adenomas and matched controls were selected for a previous study of inflammation conducted within the CLUE II cohort, and these selection methods have been previously described in detail [26]. Briefly, CLUE II participants were asked in the follow-up questionnaires if they had ever undergone a colonoscopy or sigmoidoscopy, and if so, whether a polyp was diagnosed. After obtaining permission, diagnoses were confirmed through medical record review, and cases were restricted to those with a first diagnosis of an adenomatous polyp after cohort enrollment in 1989, with no history of ulcerative colitis. A total of 135 cases of colorectal adenomatous polyps were confirmed, 123 of whom had blood available for the current analysis of JCV seroreactivity. For each case, adenoma size (in cm) and site (distal colon, proximal colon or rectum) were abstracted from the endoscopy report, and histology (villous, tubulovillous or tubular) was obtained from pathology reports. Controls for the adenoma cases were selected from CLUE II participants who reported having an endoscopy after 1989, but also reported that no polyps were detected. Controls could have no history of cancer (except NMSC or cervical cancer in situ) or self-reported polyp diagnosis through the end of follow-up. One control was matched to each adenoma case on age, race, sex, date of blood draw, date of endoscopy within 1 year, and region of the colon visualized on endoscopy (i.e. for cases with a polyp in the proximal colon, matched controls had to have had a negative colonoscopy; cases with a polyp in the distal colon could have been matched with controls who had a negative colonoscopy or sigmoidoscopy) [26].

Laboratory methods

Serum and plasma samples were shipped from the George W. Comstock Center for Public Health Research and Prevention in Hagerstown, MD to the Johns Hopkins School of Medicine in Baltimore, MD for measurement of antibodies to the JCV capsid protein, VP1. JCV virus-like-particles (VLP) were produced using a recombinant baculovirus expressing JCV VP1

[27], the amino acid sequence for which is from the NCBI reference genome (accession number NC 001699). Antibodies to VLP's were detected using enzyme immunoassays (EIA). Serum samples were diluted 1:200 and left to react on antigen coated plates for 1 hour at 37°C, after which antigen-bound immunoglobulin was detected with peroxidase-conjugated antibodies against human IgG. Color development was initiated by the addition of 2,2′-azino-di-(3-ethylbenzthiazoline-6-sulfonate) hydrogen peroxide solution. The reaction was stopped after 20 min by addition of 1% dodecyl sulfate and optical density (OD) was measured at 405 nm, with a reference wavelength of 490 nm. Each case and its matched control were maintained as a set to ensure simultaneous processing, and laboratory personnel were masked as to the case-control status of each sample. All samples were tested in duplicate, and the mean value was used in the analysis. Masked quality controls samples were included to confirm the reliability of the assay.

A cutoff for JCV seropositivity was calculated by comparing the distribution of absorbance values in the study population with the distribution of absorbance values obtained from children, ages 1 to 5 years (n=47). Young children are considered a low prevalence population for exposure to JCV, given that initial infection occurs in late childhood to early adolescence. We used an iterative statistical approach that excluded outliers in the distribution of children's test results until no remaining value was greater than three standard deviations above the mean optical density or a maximum of three iterations was reached. Seropositivity was then defined as four standard deviations above the final mean OD (minus children outliers) (absorbance value = 0.067). Using this approach, the seroprevalence in children 1–5 years of age was 10.6%.

Statistical analysis

Baseline characteristics were compared between cases and matched controls using McNemar's test for 2-level categorical variables and Bowker's test of symmetry for 3-level categorical variables. Factors assessed at baseline in both cohorts (sex, age and smoking status (current vs. never; former vs. never) and use of non-steroidal anti-inflammatory drugs (NSAIDs) within the last 48 hours) were compared between JCV-positive and JCV-negative controls using generalized linear models to account for intra-individual correlations in the subset of controls (n=118) who participated in both cohorts and contributed two observations to the analysis. Baseline body mass index (BMI) and recent hormone use in women were compared between JCV-positive and JCV-negative controls using the Fisher's exact test, as these variables were ascertained only for CLUE II participants. Associations with baseline JCV seropositivity were also compared between individuals with and without a family history of CRC, which was assessed in the CLUE II follow-up questionnaires.

Continuous JCV antibody levels were first compared between CRC cases and matched controls and colorectal adenoma cases and matched controls using the Wilcoxon Mann Whitney test. Individuals were then classified as JCV-positive or JCV-negative based on the binary cutpoint described above. The associations between JCV seropositivity and CRC or adenomas were evaluated by calculating odds ratios (OR) and 95% confidence intervals (CI) using conditional logistic regression models with robust sandwich estimates of the covariance matrix [28]. Associations between JCV and CRC were similar between the two cohorts; therefore, all observations were combined in the final analyses. For CRC, OR's were estimated overall and by sex, site (colon versus rectal cancer) stage at diagnosis, and time between blood draw and diagnosis (<1–9, 10–19 and 20–31 years), the latter for which participants in both cohorts contributed two observations in different categories. For adenomas, OR's were estimated by number of adenomas (one versus multiple), site (rectum, distal or proximal colon), histology (tubular or tubulovillous/villous) and size (< or ≥0.55 cm, the median, which was used as the cutpoint due to small numbers of cases with adenomas >1 cm). If a case had multiple adenomas, the largest adenoma and the adenoma with the worst histology were selected for analyses

conducted by size and histology, respectively. For the analysis by site, cases with multiple adenomas contributed one observations for each site at which he or she had an adenoma. Matched OR's and 95% CI's were first obtained from a model that included JCV serostatus as the only independent variable. Among CLUE II participants, a multivariable model was used to further adjust for two factors associated with JCV infection, smoking status and BMI, the latter of which was available only for CLUE II participants. Interactions between JCV seropositivity and age or sex in relation to CRC or adenoma risk were evaluated by including an interaction term in the conditional logistic regression model, the coefficient for which was evaluated by the Wald test. All statistical tests were two-sided. Analyses were conducted using SAS, version 9.1 (SAS Institute, Inc., Cary, NC).

RESULTS

Baseline characteristics of 611 CRC case-control pairs and 123 adenoma case-control pairs are presented in Table 1. Cases were more likely to be current or former smokers than their matched controls, but the differences were not statistically significant (p= 0.18 for CRC, p=0.08 for adenomas). NSAID use at baseline was less common among CRC cases versus controls (p=0.05) but did not differ between adenoma cases versus controls (p=0.18). No case-control differences were observed in BMI, while family history of CRC was statistically significantly positively associated with both CRC and adenomas (Table 1).

No difference in JCV seroprevalence was observed between males and females (Table 2). JCV seroprevalence tended to decrease with age, although this trend was not statistically significant. Former smokers were less likely to have antibodies to JCV than current and never smokers. No difference in JCV seroprevalence was observed by NSAID use at baseline. Within the CLUE II cohort, JCV seroprevalence decreased with increasing BMI at baseline (p=0.05). JCV infection was not associated with family history of CRC or recent hormone use among women (Table 2).

Results for the association between JCV infection and CRC are presented in Table 3. Based on 729 pairs of observations from 611 case-control matched pairs, JCV seropositivity was not associated with an increased risk of developing CRC (odds ratio (OR)=0.90; 95% confidence interval (CI)=0.79–1.03). The risk estimate was almost identical after adjustment for smoking and BMI among the 257 case-control pairs in the CLUE II cohort (OR = 0.94; 95% CI = 0.72–1.21). After stratification by gender, no association between JCV seropositivity and CRC was observed among men, with or without adjustment for smoking and BMI. JCV seropositivity was associated with a statistically significant decreased risk of CRC among women, although this association was attenuated after adjustment for smoking and BMI among CLUE II participants. When analyses were conducted separately for colon versus rectal cancer, no association with JCV seropositivity was observed for colon cancer, while a non-statistically significant inverse association was observed for rectal cancer. No clear patterns in JCV-associated colorectal cancer risk were observed across categories of stage at cancer diagnosis or time between blood draw and cancer diagnosis (Table 3). When JCV antibody levels were treated as a continuous variable, there were no case-control differences for colon cancer (p=0.46 for CLUE I, p=0.85 for CLUE II) or rectal cancer (p=0.31 for CLUE I, p=0.57 for CLUE II) (data not shown).

Results for the association between JCV seropositivity and colorectal adenoma are presented in Table 4. Overall, JCV seropositivity at baseline was not associated with an increased risk of adenoma development in the subsequent 15 years of follow-up. Increased risk estimates were observed for developing multiple adenomas (OR=1.81, 95% CI=0.88–3.74) and adenomas 0.55 cm or larger (OR=1.65, 95% CI+0.86–3.19), but neither of these associations were statistically significant. No clear patterns in adenoma risk were observed by location

within colon or histology. Adenoma results are stratified by gender in Table 5. JCV seropositivity at baseline was associated with a greater than twofold increased risk of subsequent adenoma among men with adjustment for smoking and BMI (OR=2.31, 95% CI=1.20–4.46). Conversely, a statistically significant decreased risk of adenoma was associated with baseline JCV seropositivity among women (OR=0.31, 95% CI=0.14–0.67) ($p<0.001$ for interaction between men and women). The positive association between JCV seropositivity and adenoma risk observed among men was particularly strong for those who developed multiple adenomas (OR=6.71, 95% CI=1.34–33.60) and for men whose largest adenoma was ≥ 0.55 cm (OR=3.83, 95% CI=1.20–12.25), the median adenoma size in this study population. Among women, JCV seropositivity was consistently associated with decreased risks of adenoma development across adenoma number and size. No clear differences in adenoma risks were observed by location within the colon versus rectum or by histology among men or women (Table 5). When JCV antibody levels were treated as a continuous variable, female adenoma cases had statistically significantly lower levels than controls ($p=0.02$). Male adenoma cases had higher JCV antibody levels than controls, although the difference was not statistically significant ($p=0.11$).

DISCUSSION

JCV seropositivity was not associated with increased risk of developing CRC up to 31 years later in either men or women. A positive association was observed between JCV seropositivity and adenoma in men, whereas an inverse association was observed in women. Our findings are consistent with the only other serological study of JCV infection and CRC, a prospective study conducted among men in Norway, which observed no increased risk of CRC among men who were JCV seropositive at baseline[25]. This present study is the first analysis of JCV antibodies in relation to CRC in women and adenomas in both men and women.

Other than age, there are no known risk factors for primary infection with JCV. Analysis of JCV antibody data from the controls in this study suggested that smoking status and BMI could also be related to JCV infection. However, adjustment for these factors did not change the association between JCV seropositivity and CRC. C-reactive protein (CRP) levels, a marker of inflammation, were available from a previous case-control study conducted within this cohort [26,29]. Adjustment for CRP levels in the subset of participants for whom data were available did not change the risk estimates for JCV seropositivity and CRC or adenomas (data not shown). JCV reactivation is common in pregnancy [30], and parity has been associated with a decreased risk of CRC in some studies [31]. However, only 8% of women had never been pregnant at baseline, and no association was observed with JCV seropositivity (data not shown). Therefore, the inverse associations observed between JCV seropositivity and CRC/adenomas among women was not likely due to negative confounding by parity. Alternative explanations for the observed differences in JCV-associated adenoma risk by gender are unclear. Increased BMI has been shown to be more strongly associated with CRC among men [32], and postmenopausal women with no recent use of hormone replacement therapy [33], suggesting estrogen status may be a modifying factor. However, there were no differences in JCV-associated adenoma risk observed between women who reported hormone use at baseline versus those who did not (data not shown).

It has been suggested that JCV may contribute to colon cancer development through a “hit-and-run” mechanism [34] whereby JCV infection is involved in the early stages of CRC carcinogenesis through disruption of the Wnt signaling pathway but is not needed for tumor progression. Specifically, co-expression of JCV T-antigen and B-catenin can result in increased transcription of c-myc[14], leading to chromosomal instability, which can progress in the absence of JCV T-antigen expression [15]. Disruption of the Wnt signaling pathway can result in chromosomal instability within normal colon mucosa and transformation to early

adenoma[35], while subsequent progression of early adenoma through intermediate and late stages and onto carcinoma is dependent upon subsequent genetic alterations. This model could explain the positive association between JCV antibodies and adenomas among men, juxtaposed with the null association with CRC. However, if JCV is involved in the earliest stages of CRC carcinogenesis, then the association between JCV seroreactivity and CRC would most likely be evident in blood samples obtained decades prior to CRC diagnosis, and no increased risk of CRC was observed in association with JCV antibodies measured up to 31 years prior to diagnosis among men (data not shown).

JCV infection elicits the formation of several types of antibodies in humans. The present study measured IgG antibodies to the JCV capsid, which are produced in response to initial asymptomatic infection with JCV, usually occurring in late childhood. The presence of JCV IgG capsid antibodies does not protect against reactivation of infection [3], although, high versus low levels of JCV capsid antibodies can distinguish people shedding virus in their urine from non-shedders, and thus, may serve as a more specific marker of JCV reactivation [25]. We compared continuous JCV IgG antibody levels between cases and controls and observed no association with CRC in males or females. However, measurement of other classes of antibodies to the JCV capsid and/or antibodies to the T-antigen may provide additional information about the association between JCV infection and cancer. Ideally, one would investigate the full signature of JCV by measuring DNA sequences and protein expression in tumor tissue, in addition to circulating antibodies [36]. However, tumor tissues were not available from the cases in this study.

The present study has additional some limitations, including the initial assessment of adenomas through self-report. The adenoma cases and controls were ascertained among respondents to the CLUE II follow-up questionnaire(s), with response rates ranging from 62% to 70%. However, it is unlikely that respondents differed from non-respondents with respect to JCV serostatus, and therefore, selection bias did not likely result. Only those adenoma cases that were verified with a pathology report were included in the current analysis. However, pathology reports were not obtained from respondents who reported having had a colonoscopy or sigmoidoscopy without an adenoma diagnosis. Therefore, it is possible that some adenoma "cases" were misclassified as "controls", potentially biasing the observed results toward the null.

Prospective studies with long duration of follow-up are important for investigations of risk factors that may be involved in the early stages of CRC carcinogenesis, such as JCV infection. To our knowledge, the present study was the first to investigate JCV infection and CRC among women, and the first seroepidemiologic study of JCV antibodies and adenomas. The sample size for the analysis of adenomas was smaller than that for CRC, and future studies are needed to replicate the findings for adenomas and to investigate mechanisms that could differ by gender. If JCV infection is indeed confirmed as a risk factor for adenomas among men, then it could be a target for novel CRC prevention strategies. However, given the inconsistencies in tumor studies, and the limited data from epidemiologic studies, more information is needed to evaluate the association.

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Table 1

Baseline characteristics of colorectal cancer cases, colorectal adenoma cases and controls, Washington County, MD, 1975 to 2006

	Colorectal cancer (CRC)					Colorectal adenomas				
	cases		controls			cases		controls		
Characteristic	**n**	**%**	**n**	**%**	**p-value**	**n**	**%**	**n**	**%**	**p-value**
Cohort participation[1]										
CLUE I only (1974)	354	57.9	354	57.9		0	0.0	0	0.0	
CLUE II only (1989)	139	22.7	139	22.7		123	100.0	123	100.0	
CLUE I and II	118	19.3	118	19.3	matched	0	0.0	0	0.0	matched
Age in years (mean +/-SD)[1]	56.2 +/− 12.4		56.2 +/− 12.3		matched	55.1 +/− 9.7		54.9 +/− 9.6		matched
Sex[1]										
male	269	44.0	269	44.0		61	49.6	61	49.6	
female	342	56.0	342	56.0	matched	62	50.4	62	50.4	matched
Race[1]										
White	600	98.2	600	98.2		123	100.0	123	100.0	
other	11	1.8	00	1.8	matched	0	0.0	0	0.0	matched
Smoking status										
current	158	21.7	154	21.1		21	17.1	15	12.2	
former	230	31.6	197	27.0		52	42.3	41	33.3	
never	341	46.8	378	51.9	0.18	50	40.7	67	54.5	0.08
Body mass index (kg/m^2)[3]										
< 25	102	39.7	104	40.5		52	42.3	51	41.5	
25–30	103	40.1	110	42.8		49	39.8	54	43.9	
30+	52	20.2	43	16.7	0.51	22	17.9	18	14.6	0.71
NSAID use at baseline[2]										
Yes	176	24.1	209	28.7		38	30.9	29	23.6	
No	553	75.9	520	71.3	0.05	85	69.1	94	76.4	0.18
Family history of CRC[3]										
no	124	78.0	150	86.7		86	71.7	98	83.8	

	Colorectal cancer (CRC)					Colorectal adenomas				
	cases		controls			cases		controls		
Characteristic	**n**	**%**	**n**	**%**	**p-value**	**n**	**%**	**n**	**%**	**p-value**
yes	35	22.0	23	13.3	0.04	34	28.33	19	16.2	0.03
Recent hormone use[3,4]										
none	178	87.7	185	89.4		49	81.7	42	67.7	
any	25	12.3	22	10.6	0.64	11	18.3	20	32.3	0.10

[1] matching factors

[2] non-steroidal anti-inflammatory drugs (NSAID) use within 48 hours prior to blood draw

[3] data obtained from CLUE II participants only

[4] data presented for females only

Table 2

Factors associated with JC virus (JCV) infection among controls, Washington County, MD, 1975 to 2006

	JCV-positive		JCV-negative		
Characteristic	**n**	**%**	**n**	**%**	**p-value**
Sex					
male	266	70.0	114	30.0	
female	341	72.2	131	27.8	0.48
Age (years)					
< 35	30	83.3	6	16.7	
35–44	67	72.8	25	27.2	
45–54	173	70.9	71	29.1	
55–64	172	67.5	83	32.5	
65–74	123	73.2	45	26.8	
75+	34	69.4	15	30.6	0.09
Smoking status					
current	125	74.0	44	26.0	0.87[1]
former	156	65.5	82	34.5	0.03[1]
never	326	73.3	119	26.7	
Body mass index (kg/m^2)[2]					
< 25	91	58.7	64	41.3	
25–30	81	49.4	83	50.6	
30+	25	41.0	36	59.0	0.05
NSAID use at baseline (past 48 hours)[3]					
yes	174	28.7	64	26.1	
no	433	74.3	181	73.9	0.45
Family history of colorectal cancer[2]					
no	127	51.2	121	48.8	
yes	22	52.4	20	47.6	1.00
Recent hormone use[2]					
none	83	50.0	83	50.0	
any	19	54.3	16	45.7	0.71

[1] p-values correspond to comparison between current versus never smokers and former versus never smokers

[2] data obtained from CLUE II participants only

[3] NSAID use within 48 hours prior to blood draw

Table 3

Prediagnostic antibodies to JC virus (JCV) and colorectal cancer, Washington County, MD, 1975–2006

	Matched analysis[1]						Multivariable model[2]	
	cases		controls					
JCV serostatus	n	%	n	%	odds ratio	95% CI	odds ratio	95% CI
Overall:								
JCV-negative	202	27.7	185	25.4	1.00	reference	1.00	reference
JCV-positive	527	72.3	544	74.6	0.90	(0.79–1.03)	0.94	(0.72–1.21)
By sex:								
males								
JCV-negative	84	26.3	84	26.3	1.00	reference	1.00	reference
JCV-positive	235	73.7	235	73.7	1.00	(0.81–1.24)	0.89	(0.59–1.34)
females								
JCV-negative	118	28.8	101	24.6	1.00	reference	1.00	reference
JCV-positive	292	71.2	309	75.4	0.84	(0.71–0.99)	0.96	(0.68–1.35)
By disease site:								
colon cancer								
JCV-negative	149	27.8	142	26.5	1.00	reference	1.00	reference
JCV-positive	387	72.2	394	73.5	0.94	(0.81–1.10)	1.00	(0.74–1.34)
distal colon[3]								
JCV-negative	59	29.2	49	24.3	1.00	reference	1.00	reference
JCV-positive	143	70.8	153	75.7	0.81	(0.64–1.02)	0.66	(0.39–1.11)
proximal colon[4]								
JCV-negative	76	25.8	85	28.8	1.00	reference	1.00	reference
JCV-positive	219	74.2	210	71.2	1.15	(0.94–1.41)	1.43	(0.96–2.12)
rectal cancer								
JCV-negative	53	27.5	43	22.3	1.00	reference	1.00	reference
JCV-positive	140	72.5	150	77.7	0.77	(0.58–1.03)	0.69	(0.38–1.27)
By stage at diagnosis:								
Local								
JCV-negative	114	30.2	115	30.5	1.00	reference	1.00	reference
JCV-positive	263	69.8	262	69.5	1.01	(0.88–1.16)	1.10	(0.80–1.52)

	Matched analysis[1]						Multivariable model[2]	
	cases		controls					
JCV serostatus	**n**	**%**	**n**	**%**	**odds ratio**	**95% CI**	**odds ratio**	**95% CI**
Regional								
JCV-negative	52	28.4	40	21.9	1.00	reference	1.00	reference
JCV-positive	131	71.6	143	78.1	0.75	(0.59–0.97)	0.66	(0.39–1.13)
Distant								
JCV-negative	25	22.7	24	21.8	1.00	reference	1.00	reference
JCV-positive	85	77.3	86	78.2	0.96	(0.69–1.33)	0.68	(0.23–2.02)
By time between blood draw and diagnosis[5]:								
<1–9 years								
JCV-negative	94	34.4	82	30.0	1.00	reference	1.00	reference
JCV-positive	179	65.6	191	70.0	0.79	(0.61–1.03)	0.77	(0.55–1.08)
10–19 years								
JCV-negative	85	29.6	89	31.0	1.00	reference	1.00	reference
JCV-positive	202	70.4	198	69.0	1.11	(0.81–1.53)	1.32	(0.85–2.06)
20–31 years								
JCV-negative	23	13.6	15	8.9	1.00	reference	-	-
JCV-positive	146	86.4	154	91.1	0.60	(0.36–1.01)	-	-

[1] Cases and controls matched on age, sex, race, cohort, and date of blood draw

[2] Results presented for CLUE II participants only; conditional logistic regression model included baseline smoking status and body mass index

[3] Distal includes the descending and sigmoid colon

[4] Proximal includes the cecum, ascending colon, hepatic flexure, transverse colon, and splenic flexure

[5] 118 case-control pairs who participated in both CLUE cohorts contributed observations to two different strata for the simple models; only their CLUE II observations were included in the multivariable model

Table 4

Prediagnostic antibodies to JC virus (JCV) and colorectal adenomas, Washington County, MD, 1989–2006

	cases		controls					
JCV serostatus	**n**	**%**	**n**	**%**	**OR[1]**	**95% CI[1]**	**OR[2]**	**95% CI[2]**
JCV-negative	65	52.9	60	48.8	1.00	reference	1.00	reference
JCV-positive	58	47.2	63	51.2	0.83	(0.57–1.22)	0.84	(0.56–1.27)
By number of adenomas:								
One adenoma								
JCV-negative	38	59.4	28	43.8	1.00	reference	1.00	reference
JCV-positive	26	40.6	36	56.3	0.52	(0.31–0.90)	0.74	(0.26–0.85)
Multiple adenomas								
JCV-negative	27	45.8	32	56.2	1.00	reference	1.00	reference
JCV-positive	32	54.2	27	45.8	1.56	(0.85–2.85)	1.81	(0.88–3.74)
By size:								
<0.55cm								
JCV-negative	29	60.4	21	43.8	1.00	reference	1.00	reference
JCV-positive	19	39.8	27	56.3	0.43	(0.21–0.89)	0.44	(0.22–0.90)
>=0.55cm								
JCV-negative	23	47.9	29	60.4	1.00	reference	1.00	reference
JCV-positive	25	52.1	19	39.6	1.67	(0.91–3.04)	1.65	(0.86–3.19)
By site:								
Rectum								
JCV-negative	12	46.2	9	34.6	1.00	reference	1.00	reference
JCV-positive	14	53.9	17	65.4	0.63	(0.28–1.41)	0.60	(0.25–1.42)
Distal[3]								
JCV-negative	38	55.1	37	56.6	1.00	reference	1.00	reference
JCV-positive	51	44.9	32	46.4	0.93	(0.56–1.56)	0.85	(0.46–1.54)
Proximal[4]								
JCV-negative	27	50.0	29	53.7	1.00	reference	1.00	reference
JCV-positive	27	50.0	25	46.3	1.20	(0.66–2.18)	1.21	(0.66–2.23)
By Histology:								
Tubular								

	cases		controls					
JCV serostatus	**n**	**%**	**n**	**%**	**OR[1]**	**95% CI[1]**	**OR[2]**	**95% CI[2]**
JCV-negative	40	54.8	34	46.6	1.00	reference	1.00	reference
JCV-positive	33	45.2	39	53.4	0.67	(0.39–1.13)	0.61	(0.36–1.04)
Tubulovillous & Villous								
JCV-negative	24	50.0	24	50.0	1.00	reference	1.00	reference
JCV-positive	24	50.0	24	50.0	1.00	(0.57–1.76)	1.16	(0.58–2.35)

[1] Cases and controls matched on age, sex, race, cohort, date of blood draw

[2] Cases and controls matched on age, sex, race, cohort, date of blood draw; conditional logistic regression model included baseline smoking status (current/former/never) and body mass index

[3] Distal includes the descending and sigmoid colon

[4] Proximal includes the cecum, ascending colon, hepatic flexure, transverse colon, and splenic flexure

[5] Not estimable

Table 5

Prediagnostic antibodies to JC virus (JCV) and colorectal adenomas by gender, Washington County, MD, 1989–2006

	males								females							
	cases		controls						cases		controls					
JCV serostatus	**n**	**%**	**n**	**%**	**OR[1]**	**95% CI[1]**	**OR[2]**	**95% CI[2]**	**n**	**%**	**n**	**%**	**OR[1]**	**95% CI[1]**	**OR[2]**	**95% CI[2]**
JCV-negative	20	32.8	30	49.2	1.00	reference	1.00	reference	45	72.6	30	48.4	1.00	reference	1.00	reference
JCV-positive	41	67.2	31	50.8	2.25	(1.20–4.23)	2.31	(1.20–4.46)	17	27.4	32	51.6	0.32	(0.16–0.63)	0.31	(0.14–0.67)
By number of adenomas:																
One adenoma																
JCV-negative	11	39.3	12	42.9	1.00	reference	1.00	reference	27	75.0	16	44.4	1.00	reference	1.00	reference
JCV-positive	17	60.7	16	57.1	1.17	(0.54–2.53)	1.37	(0.48–3.88)	9	25.0	20	55.6	0.27	(0.11–0.66)	0.11	(0.03–0.40)
Multiple adenomas																
JCV-negative	9	27.3	18	54.6	1.00	reference	1.00	reference	18	69.2	14	53.9	1.00	reference	1.00	reference
JCV-positive	24	72.7	15	45.5	5.50	(1.51–20.08)	6.71	(1.34–33.60)	8	30.8	12	46.2	0.43	(0.15–1.20)	0.40	(0.09–1.90)
By size:																
<0.55cm																
JCV-negative	10	47.6	9	42.9	1.00	reference	1.00	reference	19	70.4	12	44.4	1.00	reference	1.00	reference
JCV-positive	11	52.4	12	57.1	0.75	(0.26–2.19)	0.58	(0.20–1.67)	8	29.6	15	55.6	0.30	(0.11–0.85)	0.30	(0.12–0.74)
>=0.55cm																
JCV-negative	6	22.2	15	55.6	1.00	reference	1.00	reference	17	81.0	14	66.7	1.00	reference	1.00	reference
JCV-positive	21	77.8	12	44.4	4.00	(1.41–11.35)	3.83	(1.20–12.25)	4	19.1	7	33.3	0.50	(0.18–1.41)	0.56	(0.20–1.56)
By site:																
Rectum																
JCV-negative	3	20.0	6	40.0	1.00	reference	1.00	reference	9	81.8	3	27.3	1.00	reference	1.00	reference
JCV-positive	12	80.0	9	60.0	2.50	(0.71–8.83)	2.69	(0.87–8.27)	2	18.2	8	72.7	0.12	(0.00–0.85)	NE[5]	NE[5]
Distal[3]																
JCV-negative	13	37.1	19	54.3	1.00	reference	1.00	reference	25	73.5	18	52.9	1.00	reference	1.00	reference
JCV-positive	22	62.9	16	45.7	2.50	(1.03–6.10)	2.38	(0.89–6.38)	9	26.5	16	47.1	0.36	(0.15–0.89)	0.31	(0.11–0.87)
Proximal[4]																
JCV-negative	7	26.9	13	50.0	1.00	reference	1.00	reference	20	71.4	16	57.1	1.00	reference	1.00	reference
JCV-positive	19	73.1	13	50.0	4.00	(1.12–14.35)	5.31	(0.87–32.27)	8	28.6	12	42.9	0.50	(0.20–1.22)	0.45	(0.15–1.35)

	males								females							
	cases		controls						cases		controls					
JCV serostatus	**n**	**%**	**n**	**%**	**OR[1]**	**95% CI[1]**	**OR[2]**	**95% CI[2]**	**n**	**%**	**n**	**%**	**OR[1]**	**95% CI[1]**	**OR[2]**	**95% CI[2]**
By Histology:																
Tubular																
JCV-negative	11	30.6	16	44.4	1.00	reference	1.00	reference	29	78.4	18	48.7	1.00	reference	1.00	reference
JCV-positive	25	69.4	20	55.6	2.25	(0.92–5.49)	1.97	(0.83–4.69)	8	21.6	19	51.4	0.21	(0.08–0.61)	0.19	(0.06–0.56)
Tubulovillous & Villous																
JCV-negative	9	36.0	14	56.0	1.00	reference	1.00	reference	15	65.2	10	43.5	1.00	reference	1.00	reference
JCV-positive	16	64.0	11	44.0	2.25	(0.92–5.49)	2.90	(0.75–11.21)	8	34.8	13	55.5	0.38	(0.13–1.05)	0.33	(0.07–1.65)

[1] Cases and controls matched on age, sex, race, cohort, date of blood draw

[2] Cases and controls matched on age, sex, race, cohort, date of blood draw; conditional logistic regression model included baseline smoking status (current/former/never) and body mass index

[3] Distal includes the descending and sigmoid colon

[4] Proximal includes the cecum, ascending colon, hepatic flexure, transverse colon, and splenic flexure

[5] Not estimable