Prospective Study of JC Virus Seroreactivity and the Development of Colorectal Cancers and Adenomas

Dana E. Rollison,[1] Kathy J. Helzlsouer,[2,3] Ji-Hyun Lee,[1] William Fulp,[1] Sandra Clipp,[3] Judy A. Hoffman-Bolton,[3] Anna R. Giuliano,[1] Elizabeth A. Platz,[3] and Raphael P. Viscidi[4]

[1]Risk Assessment, Detection and Intervention, H. Lee Moffitt Cancer Center and Research Institute Tampa, Florida; and [2]Center for Prevention and Research, Mercy Medical Center, [3]Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, and [4]Department of Pediatrics, Johns Hopkins School of Medicine, Baltimore, Maryland

Abstract

Background: **Infection with JC virus has been proposed as a risk factor for colorectal cancer. A nested case-control study was conducted to evaluate the association between prediagnostic JC virus antibodies and the risk of incident colorectal cancer 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 colorectal cancer 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 JC virus were measured using virus-like particle ELISA. Associations between JC virus seropositivity and colorectal cancer and adenomas were estimated using conditional logistic regression.**

Results: **Overall, there was no association between antibodies to JC virus and colorectal cancer [odds ratio (OR), 0.91; 95% confidence interval (95% CI), 0.71-1.17]. However, a statistically significant positive association between JC virus seropositivity and subsequent adenoma diagnosis was observed among males (OR, 2.31; 95% CI, 1.20-4.46), whereas 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, JC virus seropositivity was not associated with colorectal cancer development up to 31 years later. Future studies are needed to confirm the adenoma findings.** (Cancer Epidemiol Biomarkers Prev 2009;18(5):1515–23)

Introduction

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

Several laboratory studies suggest that JC virus play a role in carcinogenesis. JC virus encodes a nonstructural 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 JC virus T-antigen in the disruption of the Wnt signaling pathway implicated in colorectal cancer carcinogenesis (13-15); coexpression of β-catenin and JC virus T-antigen results in increased transcription of c-myc (14), and JC virus–transfected cells exhibit nuclear accumulation of β-catenin and chromosomal aberrations, specifically when JC virus T-antigen is expressed (15).

Given the widespread exposure to JC virus and the oncogenic potential of the virus, several laboratories have

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Requests for reprints: Dana Rollison, 12902 Magnolia Drive Tampa, FL 33612. Phone: 813-745-6530; Fax: 813-745-6525. E-mail: dana.rollison@moffitt.org

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

To further investigate the potential role of JC virus in colorectal cancer, a prospective study of JC virus antibodies and incident colorectal cancer and adenomas was conducted in males and females.

Materials and 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 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 colorectal cancer 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 colorectal cancer, in which colorectal cancer was their first cancer diagnosis with the possible exceptions of nonmelanoma skin cancer or cervical cancer *in situ*. Cases had to have been Washington County residents at both the time of baseline blood donation and colorectal cancer diagnosis. A total of 611 colorectal cancer 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 colorectal cancer cases). One control was matched to each colorectal cancer case on sex, race, age within 1 y, cohort (CLUE I, CLUE II, or both), and date of blood draw within 2 wk (n = 729 blood samples from 611 matched controls, including 118 controls who participated in both CLUE cohorts). Controls for the colorectal cancer 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 nonmelanoma skin cancer or cervical cancer *in situ*) as of the date of diagnosis of the case. Vital status was 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 JC virus 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 nonmelanoma skin cancer 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 y, 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; ref. 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 JC virus capsid protein, VP1. JC virus virus-like particles were produced using a recombinant baculovirus expressing JC virus VP1 (27), the amino acid sequence for which is from the National Center for Biotechnology Information (NCBI) reference genome (accession number NC 001699). Antibodies to virus-like particles were detected using enzyme immunoassays. Serum samples were diluted 1:200 and left to react on antigen-coated plates for 1 h 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 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 JC virus 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 y (n = 47). Young children are considered a low-prevalence population for exposure to JC virus, 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 SDs above the mean optical density or a maximum of three iterations was reached. Seropositivity was then defined as four SDs above the final mean optical density (minus children outliers; absorbance value = 0.067). Using this approach, the seroprevalence in children ages 1 to 5 y was 10.6%.

Statistical Analysis. Baseline characteristics were compared between cases and matched controls using McNemar's test for two-level categorical variables and Bowker's test of symmetry for three-level categorical variables. Factors assessed at baseline in both cohorts [sex, age and smoking status (current versus never; former versus never) and use of nonsteroidal anti-inflammatory drugs within the last 48 h] were compared between JC virus–positive and JC virus–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 JC virus–positive and JC virus–negative controls using Fisher's exact test, as these variables were ascertained only for CLUE II participants. Associations with baseline JC virus seropositivity were also compared between individuals with and without a family history of colorectal cancer, which was assessed in the CLUE II follow-up questionnaires.

Continuous JC virus antibody levels were first compared between colorectal cancer cases and matched

Table 1. Baseline characteristics of colorectal cancer cases, colorectal adenoma cases, and controls, Washington County, MD, 1975 to 2006

Characteristic	Colorectal cancer			Colorectal adenomas		
	Cases	Controls	P	Cases	Controls	P
	n (%)	n (%)		n (%)	n (%)	
Cohort participation*						
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 y (mean ± SD)*	56.2 ± 12.4	56.2 ± 12.3	Matched	55.1 ± 9.7	54.9 ± 9.6	Matched
Sex*						
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*						
White	600 (98.2)	600 (98.2)		123 (100.0)	123 (100.0)	
Other	11 (1.8)	11 (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) †						
<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 ‡						
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 colorectal cancer †						
No	124 (78.0)	150 (86.7)		86 (71.7)	98 (83.8)	
Yes	35 (22.0)	23 (13.3)	0.04	34 (28.3)	19 (16.2)	0.03
Recent hormone use † §						
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

Abbreviation: NSAID, nonsteroidal anti-inflammatory drugs.
*Matching factors.
†Data obtained from CLUE II participants only.
‡NSAID use within 48 h prior to blood draw.
§Data presented for females only.

Table 2. Factors associated with JC virus infection among controls, Washington County, MD, 1975 to 2006

Characteristic	JC virus–positive	JC virus–negative	*P*
	n (%)	*n* (%)	
Sex			
Male	266 (70.0)	114 (30.0)	
Female	341 (72.2)	131 (27.8)	0.48
Age (y)			
<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*
Former	156 (65.5)	82 (34.5)	0.03*
Never	326 (73.3)	119 (26.7)	
Body mass index (kg/m^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 h) ‡			
Yes	174 (28.7)	64 (26.1)	
No	433 (74.3)	181 (73.9)	0.45
Family history of colorectal cancer †			
No	127 (51.2)	121 (48.8)	
Yes	22 (52.4)	20 (47.6)	1.00
Recent hormone use †			
None	83 (50.0)	83 (50.0)	
Any	19 (54.3)	16 (45.7)	0.71

*P values correspond to comparison between current versus never smokers and former versus never smokers.
†Data obtained from CLUE II participants only.
‡NSAID use within 48 h prior to blood draw.

controls and colorectal adenoma cases and matched controls using the Wilcoxon Mann-Whitney test. Individuals were then classified as JC virus–positive or JC virus–negative based on the binary cutpoint described above. The associations between JC virus seropositivity and colorectal cancer or adenomas were evaluated by calculating odds ratios (OR) and 95% confidence intervals (95% CI) using conditional logistic regression models with robust sandwich estimates of the covariance matrix (28). Associations between JC virus and colorectal cancer were similar between the two cohorts; therefore, all observations were combined in the final analyses. For colorectal cancer, ORs 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 y), the latter for which participants in both cohorts contributed two observations in different categories. For adenomas, ORs 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 observation for each site at which the case had an adenoma. Matched ORs and 95% CIs were first obtained from a model that included JC virus serostatus as the only independent variable. Among CLUE II participants, a multivariable model was used to further adjust for two factors associated with JC virus infection, smoking status and BMI, the latter of which was available only for CLUE II participants. Interactions between JC virus seropositivity and age or sex in relation to colorectal cancer 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.).

Results

Baseline characteristics of 611 colorectal cancer 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 colorectal cancer, P = 0.08 for adenomas). Nonsteroidal anti-inflammatory drug use at baseline was less common among colorectal cancer 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, although family history of colorectal cancer was statistically significantly positively associated with both colorectal cancer and adenomas (Table 1).

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

Results for the association between JC virus infection and colorectal cancer are presented in Table 3. Based on 729 pairs of observations from 611 case-control matched pairs, JC virus seropositivity was not associated with an increased risk of developing colorectal cancer (OR, 0.90; 95% 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 JC virus seropositivity and colorectal cancer was observed among men, with or without adjustment for smoking and BMI. JC virus seropositivity was associated with a statistically significant decreased risk of colorectal cancer 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 JC virus seropositivity was observed for colon cancer, whereas a nonstatistically significant inverse association was observed for rectal cancer. No clear patterns in JC virus–associated colorectal cancer risk were observed across categories of stage at cancer diagnosis or time between blood draw and cancer

Table 3. Prediagnostic antibodies to JC virus and colorectal cancer, Washington County, MD, 1975-2006

JCV serostatus	Matched analysis*			Multivariable model†
	Cases	Controls	OR (95% CI)	OR (95% CI)
	n (%)	*n* (%)		
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‡				
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§				
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)
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:‖				
<1-9 y				
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 y				
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 y				
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)	

Abbreviation: JCV, JC virus.

*Cases and controls matched on age, sex, race, cohort, and date of blood draw.

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

‡Distal includes the descending and sigmoid colon.

§Proximal includes the cecum, ascending colon, hepatic flexure, transverse colon, and splenic flexure.

‖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.

diagnosis (Table 3). When JC virus 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 JC virus seropositivity and colorectal adenoma are presented in Table 4. Overall, JC virus 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, 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. JC virus seropositivity at baseline was associated with a >2-fold 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 JC virus 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 JC virus seropositivity and adenoma risk observed among men was particularly strong for

Table 4. Prediagnostic antibodies to JC virus and colorectal adenomas, Washington County, MD, 1989-2006

JCV serostatus	Cases	Controls	OR*(95% CI*)	OR† (95% CI†)
	n (%)	*n* (%)		
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‡				
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§				
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				
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 and 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)

*Cases and controls matched on age, sex, race, cohort, date of blood draw.
†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.
‡Distal includes the descending and sigmoid colon.
§Proximal includes the cecum, ascending colon, hepatic flexure, transverse colon, and splenic flexure.

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, JC virus 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 JC virus 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 JC virus antibody levels than controls, although the difference was not statistically significant ($P = 0.11$).

Discussion

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

Other than age, there are no known risk factors for primary infection with JC virus. Analysis of JC virus antibody data from the controls in this study suggested that smoking status and BMI could also be related to JC virus infection. However, adjustment for these factors did not change the association between JC virus seropositivity and colorectal cancer. C-reactive protein levels, a marker of inflammation, were available from a previous case-control study conducted within this cohort (26, 29). Adjustment for C-reactive protein levels in the subset of participants for whom data were available did not change the risk estimates for JC virus seropositivity and colorectal cancer or adenomas (data not shown). JC virus reactivation is common in pregnancy (30), and parity has been associated with a decreased risk of colorectal cancer in some studies (31). However, only 8% of women had never been pregnant at baseline, and no association was observed with JC virus seropositivity

(data not shown). Therefore, the inverse associations observed between JC virus seropositivity and colorectal cancer/adenomas among women were not likely due to negative confounding by parity. Alternative explanations for the observed differences in JC virus–associated adenoma risk by gender are unclear. Increased BMI has been shown to be more strongly associated with colorectal cancer 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 JC virus–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 JC virus may contribute to colon cancer development through a ''hit-and-run'' mechanism (34) whereby JC virus infection is involved in the early stages of colorectal cancer carcinogenesis through disruption of the Wnt signaling pathway but is not needed for tumor progression. Specifically, coexpression of JC virus T-antigen and β-catenin can result in increased transcription of c-myc (14), leading to chromosomal instability, which can progress in the absence of JC virus 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), whereas 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 JC virus antibodies and adenomas among men, juxtaposed with the null association with colorectal cancer. However, if JC virus is involved at the earliest stages of colorectal cancer carcinogenesis, then the association between JC virus seroreactivity and colorectal cancer would most likely be evident in blood samples obtained decades prior to colorectal cancer diagnosis, and no increased risk of colorectal cancer was observed in association with JC virus antibodies measured up to 31 years prior to diagnosis among men (data not shown).

JC virus infection elicits the formation of several types of antibodies in humans. The present study measured

Table 5. Prediagnostic antibodies to JC virus and colorectal adenomas by gender, Washington County, MD, 1989-2006

JCV serostatus	Males				Females			
	Cases	Controls	OR* (95% CI)*	OR† (95% CI)†	Cases	Controls	OR* (95% CI)*	OR† (95% CI)†
	n (%)	*n* (%)			*n* (%)	*n* (%)		
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 (NE)
Distal‡								
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§								
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)
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 and 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)

Abbreviation: NE, not estimable.

*Cases and controls matched on age, sex, race, cohort, date of blood draw.

†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.

‡Distal includes the descending and sigmoid colon.

§Proximal includes the cecum, ascending colon, hepatic flexure, transverse colon, and splenic flexure.

IgG antibodies to the JC virus capsid, which are produced in response to initial asymptomatic infection with JC virus, usually occurring in late childhood. The presence of JC virus IgG capsid antibodies does not protect against reactivation of infection (3), although high versus low levels of JC virus capsid antibodies can distinguish people shedding virus in their urine from nonshedders, and thus, may serve as a more specific marker of JC virus reactivation (25). We compared continuous JC virus IgG antibody levels between cases and controls and observed no association with colorectal cancer in males or females. However, measurement of other classes of antibodies to the JC virus capsid and/or antibodies to the T-antigen may provide additional information about the association between JC virus infection and cancer. Ideally, one would investigate the full signature of JC virus 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 some additional 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%. It is unlikely, however, that respondents differed from nonrespondents with respect to JC virus 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 colorectal cancer carcinogenesis, such as JC virus infection. To our knowledge, the present study was the first to investigate JC virus infection and colorectal cancer among women, and the first seroepidemiologic study of JC virus antibodies and adenomas. The sample size for the analysis of adenomas was smaller than that for colorectal cancer, and future studies are needed to replicate the findings for adenomas and to investigate mechanisms that could differ by gender. If JC virus infection is indeed confirmed as a risk factor for adenomas among men, then it could be a target for novel colorectal cancer prevention strategies. However, given the inconsistencies in tumor studies, and the limited data from epidemiologic studies, more information is needed to evaluate the association.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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