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Tobacco, alcohol, and *p53* overexpression in early colorectal neoplasia

Mary Beth Terry*[1,2], Alfred I Neugut[1,2,3], Mahesh Mansukhani[4], Jerome Waye[5], Noam Harpaz[6] and Hanina Hibshoosh[4]

Address: [1]Department of Epidemiology, Columbia University Mailman School of Public Health, New York, New York, 10032 U.S.A, [2]Herbert Irving Comprehensive Cancer Center, College of Physicians and Surgeons, Columbia University, New York, NY 10032 U.S.A, [3]Department of Medicine, College of Physicians and Surgeons, Columbia University, New York, NY 10032, U.S.A, [4]Department of Pathology, College of Physicians and Surgeons, Columbia University, New York, New York, 10032 U.S.A, [5]Department of Medicine, Mt. Sinai School of Medicine, New York, New York, 10029 U.S.A and [6]Department of Pathology, Mt. Sinai School of Medicine, New York, New York, 10029 U.S.A

Email: Mary Beth Terry* - mt146@columbia.edu; Alfred I Neugut - ain1@columbia.edu; Mahesh Mansukhani - mm322@columbia.edu; Jerome Waye - jdwaye@aol.com; Noam Harpaz - nharpaz@mssm.edu; Hanina Hibshoosh - hhh1@columbia.edu

* Corresponding author

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Abstract

Background: The *p53* tumor suppressor gene is commonly mutated in colorectal cancer. While the effect of *p53* mutations on colorectal cancer prognosis has been heavily studied, less is known about how epidemiologic risk factors relate to *p53* status, particularly in early colorectal neoplasia prior to clinically invasive colorectal cancer (including adenomas, carcinoma *in situ* (CIS), and intramucosal carcinoma).

Methods: We examined *p53* status, as measured by protein overexpression, in 157 cases with early colorectal neoplasia selected from three New York City colonoscopy clinics. After collecting paraffin-embedded tissue blocks, immunohistochemistry was performed using an anti-*p53* monoclonal mouse IgG_2a [BP53-12-1] antibody. We analyzed whether *p53* status was different for risk factors for colorectal neoplasia relative to a polyp-free control group (n = 508).

Results: *p53* overexpression was found in 10.3%, 21.7%, and 34.9%, of adenomatous polyps, CIS, and intramucosal cases, respectively. Over 90% of the tumors with *p53* overexpression were located in the distal colon and rectum. Heavy cigarette smoking (30+ years) was associated with cases not overexpressing p53 (OR = 1.8, 95% CI = 1.1–2.9) but not with those cases overexpressing *p53* (OR = 1.0, 95% CI = 0.4–2.6). Heavy beer consumption (8+ bottles per week) was associated with cases overexpressing *p53* (OR = 4.0, 95% CI = 1.3–12.0) but not with cases without *p53* overexpression (OR = 1.6, 95% CI = 0.7–3.7).

Conclusion: Our findings that *p53* overexpression in early colorectal neoplasia may be positively associated with alcohol intake and inversely associated with cigarette smoking are consistent with those of several studies of *p53* expression and invasive cancer, and suggest that there may be relationships of smoking and alcohol with p53 early in the adenoma to carcinoma sequence.

Background

The molecular model of genetic changes along the colorectal adenoma-carcinoma sequence, first described by Vogelstein and colleagues, has become the framework for understanding the timing and role multiple mutations and genetic alterations play in carcinogenesis [1]. It is thought that multiple mutations are needed for invasive cancer [1–4]. Although the number of mutations and not their order has been found to be paramount, there are general trends in terms of timing in the sequence. Of more common genes important to colorectal carcinogenesis (e.g., APC, K-ras, and *p53*), most data suggest that *p53* is generally mutated later in the adenoma-carcinoma sequence [1,3–9]. The prevalence of *p53* protein overexpression, which is highly correlated with *p53* gene mutations [10–12], increases along the adenoma-carcinoma sequence with as little as 7.1 percent overexpression in adenomas with mild dysplasia to over sixty percent in those with severe dysplasia and invasive cancer [13–15].

Although the majority of colorectal cancers are thought to originate from adenomas, most adenomas do not progress to cancer. In fact, even though the prevalence of adenomas in most high-risk populations is extremely high (as high as 20 percent in people under 50 and over 50 percent among people in their 70s [16], colorectal cancer is still a rare event. Estimates of the cumulative risk of invasive cancer after adenoma detected on screening exam range from 1 to 10 percent depending on the length of follow-up [16,17]. Apart from pathologic characteristics such as larger size and villous histology, little is known about risk factors for intermediate steps along the adenoma-carcinoma sequence such as carcinoma *in situ* (CIS) and intramucosal carcinoma [18].

We undertook this investigation to examine if risk factors for early colorectal neoplasia differed by *p53* status. Specifically, we were interested in whether colorectal cancer risk factors including alcohol use and cigarette smoking are associated with *p53* status in early colorectal neoplasia and if these associations were similar to associations with invasive colorectal cancer. If so, such findings would suggest that processes contributing to the relationships between risk factors and *p53* protein overexpression begin early in the adenoma-carcinoma sequence and may point to factors to target for intervention and future prevention. We conducted two analyses, one combining all cases of early colorectal neoplasia (subjects with adenomatous polyps, carcinoma *in situ* (CIS), and intramucosal carcinoma (IM)) and a second examining only cases with advanced adenomas (subjects with CIS and IM). Together these case groups represent steps along the adenoma carcinoma sequence prior to clinically relevant invasive colorectal cancer.

Methods

Study population

The cases and controls for this study come from a case-control study of newly diagnosed adenomas and polyp-free controls conducted in three New York City colonoscopy clinics between April 1986 to March 1988 [19,20]. Both the parent study and this study were approved by Columbia's Institutional Review Board. There were 3,008 individuals who underwent colonoscopy during this time frame at the three colonoscopy clinics, of whom 2,443 (81.2%) were eligible to participate in this study. To be eligible, the subjects in this study were required to have had a complete colonoscopy; to be English- or Spanish-speaking; and to be between the ages of 35 and 84 years (18.8% of the subjects were not eligible because of incomplete colonoscopy, language restrictions and or age restrictions). Of the 2,443 eligible subjects, 2,001 subjects (81.9%) were successfully interviewed, both by telephone (71%) and by mailed questionnaire (29%) with follow-up telephone calls to complete incomplete items. The questionnaire contained information on demographics, past medical history, dietary habits, alcohol intake, smoking history, body size, physical activity, and other lifestyle factors.

The 2,001 interviewed subjects were subdivided into several categories, based on the diagnosis at the index colonoscopy and after a uniform pathologic review. Of these 2,001 subjects, there were 508 subjects free of any polyp (including adenomatous and hyperplastic) with a normal index colonoscopy and no prior history of colorectal neoplasia, 269 subjects with newly diagnosed adenoma, with no prior history of colorectal neoplasia, 57 carcinoma *in situ* (CIS) cases with or without a known history of adenomas, and 58 intramucosal carcinoma cases with or without a known history of adenomas (n = 58). The remaining subjects (n = 1,109) had pathologic diagnoses that were not of interest to this current investigation including hyperplastic polyps. For this study of early colorectal neoplasia, we collected paraffin-embedded tumor tissue blocks for the cases with intramucosal carcinoma, carcinoma *in situ* (CIS) and a random sample of cases with adenomatous polyps. Blocks with enough tissue for immunohistochemistry were retrieved for 46 (80%) of the CIS cases, 43 (75%) of the IM cases and a random sample of 25% of the adenomatous polyp cases (n = 68) frequency-matched by age (within 5 years) and sex to the other case groups. Risk factor data were not statistically significantly different between those with tumor blocks available and those without (data not shown). The cases were compared with polyp-free controls (n = 508).

Immunohistochemistry

Immunohistochemistry[21] was performed on using five :m formalin-fixed, paraffin-embedded tissue sections,

placing them on silane-coated slides and baking at 60° for 30 minutes. Afterwards, the slides are de paraffinized, hydrated, place in 10 mM citrate buffer (pH 6) and microwaved for a total of ten minutes (antigen retrieval). Appropriate blocking serum (Horse serum) and an anti-*p53* monoclonal Mouse IgG2a [BP53-12-1] antibody (1:300 dilution, BioGenex, San Ramon, CA) was used. Detection method used Vectastain, Elite ABC kit (Vector Laboratory, Burlington, CA). Chromogen diamino-benzidine was used and sections were counter-stained with methyl green (Ethyl Green; Sigma Chemical Co; St. Louis MO). Nuclear staining of tumor tissue, from a single slide, was evaluated by a semiquantitative scoring system for intensity and percent positive nuclei. A positive control with known strongly positive *p53* staining was used for comparison in each batch. Adjacent non-neoplastic colonic epithelium was used as a negative control for each batch. Two study pathologists (HH and MM) blinded to case status reviewed the stained slides and scored each case based on staining intensity and percent of cells on slide showing evidence of overexpression. Disagreements in ratings were resolved by consensus of the two pathologists. The following categories were used for scoring: intensity (None, Mild, Moderate, and Strong) and percent positive (none or rare, < 10% nuclear staining, 10–25%, 25–50%, and >50%). Cutoff levels reflect levels of staining not observed in normal colonic mucosa controls. Cases were classified as positive if the intensity score was strong and at least 10% or more of cells showing evidence of overexpression. For ease of presentation, we refer to these cases with protein overexpression as *p53*+ cases throughout the manuscript; those cases with no, mild or moderate staining intensity with any percent positive or those with strong staining but less than 10% positive nuclear staining are referred to as *p53*- cases. We also performed sensitivity analyses on the final models using a more stringent (intensity score strong and at least 25% or more of cells showing evidence of overexpression) as well as a less stringent definition (intensity score of moderate or strong with at least 10% or more of cells showing evidence of overexpression) of positivity.

Statistical methods

First, univariate analyses comparing epidemiologic risk factors and *p53* status were performed using analysis of variance for continuous variables and chi-square tests for categorical variables. All continuous variables were then categorized based on quantiles in the control group to test for linearity with respect to outcome status. Second, we used unordered polytomous logistic regression models to adjust for potential confounding variables [22]. We performed two separate analyses: the first, combined all three groups (adenomas, CIS, and IM) into a single case group of early colorectal neoplasia; the second, examined only examined advanced adenomas (CIS and IM). Our sample size limited the use of multivariate models to analyze each type of case separately by *p53* status, though univariate analyses suggested that the association between risk factors and *p53* status were similar in magnitude across the separate types of cases (adenomas, CIS, and IM) (data not shown). There were a total of three outcome categories: *p53* + cases, *p53* - cases, and polyp-free controls and comparisons where made using odds ratios (OR) and 95% confidence intervals (CI) for *p53*+ cases versus polyp-free controls, *p53*- cases versus controls, and *p53*+ versus *p53*- cases.

All continuous variables that did not have a linear relationship with outcome status were categorized in categories based on homogeneity in risk [23]. To account for the non-linear relationship with age, age adjustments were made by adding age and age squared (age^2) to the regression model. Model building was based on log likelihood tests [22]. Heterogeneity by *p53* status was examined by examining the ratio of the odds ratio (which is equivalent to exponentiating the difference in the beta coefficients) and the 95% CI (based on the variance for the difference in the beta coefficients)[22].

Results

The prevalence of *p53* protein overexpression increased steadily across the adenoma-carcinoma sequence from 10.3%, 21.7%, and 34.9% for adenomatous polyps, CIS, and intramucosal carcinoma (IM), respectively. Although, *p53* protein overexpression differed by type of case group ($p < .01$), univariate associations between risk factors (including family history, alcohol use, tobacco use, body size, physical activity, dietary fiber and fat) and *p53* status did not differ in magnitude or direction for the three types of cases, albeit numbers were small. Because of these similar univariate association and because of statistical power constraints we conducted two main multivariate analyses, one combining the three types of cases (adenomas, CIS, and IM) and a second only examining the more advanced adenomas (CIS and IM).

Univariate differences in risk factors by *p53* status are reported in Table 1. In this table we combined cases of adenomatous polyps, CIS, and IM and then stratified based on *p53* status. There were no statistically significant differences in risk factors stratified by *p53* status though subjects with no overexpression tended to smoke for more years with greater intensity and subjects with overexpression tended to drink more. Over 90% of the polyps overexpressing *p53* were located in the rectum and distal colon. The comparison of *p53* status by major polyp site (rectum, distal, and proximal) was statistically significant ($p < 0.01$). The prevalence of *p53* protein overexpression by polyp site was 32%, 27%, and 6% for rectum, distal, and proximal colon, respectively.

Table 1: Descriptive Statistics by *p53* status.

	No *p53* protein overexpression (n = 125)	*p53* protein overexpression (n = 32)	P value*
Sex			
Male	66(52.8%)	16 (50%)	0.77
Female	59 (47.2%)	16 (50%)	
Age (μ μ ;$\pm$ σ)	65.7 (8.6)	67.8 (9.5)	0.23
Race			
White	103 (83.1%)	30 (93.8%)	0.24
Black	12 (9.7%)	2 (6.2%)	
Other	9 (7.3%)	0 (0%)	
Family History of Colorectal Cancer			
Yes	28 (22.4%)	5(15.6%)	0.40
No	97 (77.6%)	27 (84.4%)	
Cigarette Smoking			
Nonsmokers	43 (34.4%)	12 (38.7%)	0.90
Former Smokers	61 (48.8%)	14 (45.2%)	
Current Smokers	21 (16.8%)	5 (16.1%)	
Among smokers			
Age at Starting ($\mu \pm \sigma$)	25.3 (11.3)	29.6 (14.5)	0.17
Total years ($\mu \pm \sigma$)	29.2 (14.8)	24.6 (16.3)	0.23
Cigs per day ($\mu \pm \sigma$)	24.7 (17.2)	22.7 (20.2)	0.66
Pipe or Cigar smoking			
Never	106 (84.8%)	25 (78.1%)	0.40
Ever	19 (15.2%)	7 (21.9%)	
Alcohol intake			
# of alcoholic drinks per week ($\mu \pm \sigma$)	10.5 (15.6)	12.6 (15.8)	0.50
Other risk factors			
Body Mass Index (kg/m^2) ($\mu \pm \sigma$)	25.2 (4.3)	25.6 (5.3)	0.64
Leisure Physical Activity Hours/week ($\mu \pm \sigma$)	0.6 (2.6)	0.9 (1.9)	0.69
Daily Fiber Intake Grams/day ($\mu \pm \sigma$)	16.3 (6.9)	16.1 (6.6)	0.86
Daily Fat Intake Grams/day ($\mu \pm \sigma$)	76.4 (39.4)	69.7 (27.9)	0.40
Polyp site			
Rectum	19 (16.4%)	9 (29.0%)	<0.01
Distal	51 (44.0%)	19 (61.3%)	
Proximal	46 (39.7%)	3 (9.7%)	

* P value from analysis of variance for continuous variables and for chi-square tests for categorical variables.

Age-adjusted odds ratios from unordered polytomous logistic regression models are presented in Table 2. Long term cigarette smoking (30+ years) was associated with cases whose tumors that did not overexpress the *p53* protein (OR = 1.8, 95% CI = 1.1–2.9) but not with cases with tumors that overexpressed the *p53* protein (OR = 1.1, 95% CI 0.4–2.8), relative to polyp free controls. In contrast, heavy beer consumption (8+ bottles per week) was stronger for cases whose tumors overexpressed the *p53* protein (OR = 4.5, 95%CI = 1.6–12.4) than for those cases without *p53* protein (OR = 2.2, 95% CI = 1.0–4.7). Risk factors for colorectal neoplasia, other than alcohol and cigarette smoking, did not vary by *p53* status.

The multivariate adjusted model, reported in Table 3 (panel A), found similar associations to the age-adjusted models. Long term cigarette smokers (30+ years) had an 80% (OR = 1.8, 95% CI = 1.1–2.9) increase in risk of adenomatous polyps (with and without CIS and IM) that do not overexpress *p53* protein relative to polyp-free controls. Heavy beer consumers (8+ bottles per week) had 4 times the risk (OR = 4.0, 95% CI = 1.3–12.0) of adenomatous polyps (with and without CIS and IM) that overexpress *p53* protein relative to polyp-free controls.

We also examined these associations for only the cases of CIS and IM. These findings are also reported in Table 3 (panel B) and suggest little differences between the analyses for all three groups combined. Although these results suggest that cigarette smoking may be associated mainly with the tumors that do not overexpress *p53* protein and beer consumption mainly with the tumors that overexpress *p53* protein, statistical tests of heterogeneity were not significant (see the case/case comparisons reported in Table 3). Specifically, the comparison of heavy beer consumption for subjects with polyps that overex-

Table 2: Age adjusted odds ratios by *p53* status relative to polyp-free controls.

	No *p53* protein overexpression	***p53* protein overexpression**
Cigarette Smoking		
Never	1.0	1.0
Former	1.3 (0.8–2.1)	1.1 (0.5–2.5)
Current	1.7 (0.9–3.2)	1.6 (0.5–4.8)
Total number of years smoking*		
< 15 years	1.4 (0.7–2.7)	2.2 (0.8–6.4)
15 – 29 years	0.9 (0.5–1.7)	0.9 (0.3–2.7)
30+ years	1.8 (1.1–2.9)	1.1 (0.4–2.8)
Pipes or cigars		
Never	1.0	1.0
Ever	1.0 (0.5–1.7)	1.6 (0.7–3.9)
Alcohol		
Nondrinkers	1.0	1.0
Beer 1–7 bottles/week	1.6 (0.9–2.6)	0.4 (0.1–1.9)
Beer 8+ bottles/week	2.2 (1.0–4.7)	4.5 (1.6–12.4)
Wine (any wine during week)	1.2 (0.8–1.8)	0.8 (0.4–1.8)
Liquor (any liquor during week)	1.2 (0.8–1.8)	1.0 (1.0–4.2)
Total alcohol (wine+beer+liquor)		
1–13 drinks/week	1.1 (0.7–1.8)	0.8 (0.3–2.1)
14+ drinks/week	1.5 (0.9–2.6)	2.4 (1.0–5.7)
Body Size and Physical Activity		
Body mass index (kg/m^2)		
< 22.5	1.0	1.0
22.5 – <25.5	1.1 (0.8–2.3)	1.2 (0.5–3.7)
25.5+	2.0 (1.2–3.3)	1.7 (0.7–4.3)
Leisure physical activity		
None	1.0	1.0
2–4 hours/week	0.5 (0.2–1.4)	0.5 (0.1–3.5)
5+ hours/week	0.8 (0.4–1.6)	1.3 (0.5–3.7)
Dietary variables		
Fiber: < 12.5 grams/day	1.0	1.0
12.5 – <17 grams/day	0.9 (0.5–1.5)	0.3 (0.1–1.1)
17+ grams/day	1.1 (0.7–1.9)	0.9 (0.4–2.2)
Fat: < 60 grams/day	1.0	1.0
60 – <85.5 grams/day	0.7 (0.4–1.2)	1.6 (0.6–3.9)
85.5 grams/day	1.1 (0.7–1.8)	1.2 (0.5–3.3)

Table 3: Multivariate adjusted* odds ratios by *p53* status relative to polyp-free controls.

	No *p53* protein overexpression (-)	***p53* protein overexpression (+)**	**Case/Case comparison**
	(A) Associations using all cases (adenomas, CIS, IM)		
Duration of Cigarette Smoking			
Nonsmoker	1.0	1.0	
< 15 years	1.5 (0.7–3.0)	2.1 (0.7–6.4)	1.5 (0.4–4.9)
15 – 29 years	1.0 (0.5–1.8)	0.9 (0.3–2.8)	0.9 (0.3–3.1)
30+ years	1.8 (1.1–2.9)	1.0 (0.4–2.6)	0.6 (0.2–1.5)
Pipes or cigars			
Never	1.0	1.0	
Ever	0.6 (0.3–1.2)	1.3 (0.4–4.0)	2.1 (0.7–6.9)
Beer Consumption			
Nondrinker	1.0	1.0	
1–7 bottles/week	1.5 (0.9–2.5)	0.4 (0.1–1.9)	0.3 (0.1–1.4)
8+ bottles/week	1.6 (0.7–3.7)	4.0 (1.3–12.0)	2.5 (0.7–8.1)

Table 3: Multivariate adjusted* odds ratios by *p53* status relative to polyp-free controls. *(Continued)*

Body Size			
Body mass index (kg/m^2)			
< 22.5	1.0	1.0	
22.5 – <25.5	1.2 (0.7–2.1)	1.2(0.4–3.5)	1.1 (0.4–3.3)
25.5+	1.8 (1.0–3.1)	1.5 (0.6–4.2)	0.9 (0.3–2.5)
(B) Associations using all only cases with advanced adenomas (CIS and IM)			
Duration of Cigarette Smoking			
Nonsmoker	1.0	1.0	
< 15 years	1.1 (0.4–2.8)	2.7 (0.8–9.6)	2.5(0.6–11.7)
15 – 29 years	0.7 (0.3–1.7)	0.7 (0.2–2.7)	1.0 (0.2–4.5)
30+ years	1.9 (1.0–3.5)	0.9 (0.3–2.8)	0.5 (0.2–1.7)
Pipes or cigars			
Never	1.0	1.0	
Ever	0.5 (0.2–1.2)	1.5 (0.4–5.5)	2.9(0.6–12.9)
Beer Consumption			
Nondrinker	1.0	1.0	
1–7 bottles/week	0.9 (0.4–2.0)	0.3 (0.04–2.4)	0.3(0.04–2.9)
8+ bottles/week	1.4 (0.5–3.8)	4.1 (1.2–14.4)	3.0(0.7–13.2)
Body Size			
Body mass index (kg/m^2)			
< 22.5	1.0	1.0	
22.5 – <25.5	0.8 (0.4–1.7)	1.4(0.4–4.8)	1.7 (0.4–6.7)
25.5+	1.3 (0.7–2.6)	2.2 (0.7–7.0)	1.7 (0.5–6.1)

*age, age^2, gender, race, and the other variables listed in the table.

Table 4: Sensitivity analyses altering the definition of *p53* positivity.

	Original *a priori* definition [a]	More stringent definition [b]	Less stringent definition [c]
Long duration of Cigarette Smoking (30+ years)			
No *p53* protein overexpression	1.8 (1.1–2.9)	1.8 (1.1–2.9)	2.1 (1.1–4.3)
p53 protein overexpression	1.0 (0.4–2.6)	0.9 (0.3–2.7)	1.4 (0.8–2.4)
Case/Case Comparison	0.6 (0.2–1.5)	0.5 (0.2–1.6)	0.6 (0.3–1.4)
Heavy Beer Consumption (8 + bottles per week)			
No *p53* protein overexpression	1.6 (0.7–3.7)	1.5 (0.7–3.5)	2.4 (0.9–6.5)
p53 protein overexpression	4.0 (1.3–12.0)	4.9 (1.5–15.8)	1.8 (0.8–4.3)
Case/Case Comparison	2.5 (0.7–8.1)	3.2 (0.9–11.1)	0.8 (0.3–2.3)

[a]Original definition: intensity score of strong and at least 10% or more of cells showing evidence of overexpression [b]More stringent definition: intensity score of strong and at least 25% or more of cells showing evidence of overexpression [c]Less stringent definition: intensity score of moderate or strong and at least 10% or more of cells showing evidence of overexpression

press *p53* protein relative to those without overexpression was over two-fold but not statistically significant (OR = 2.5, 95% CI = 0.7–8.1). Comparison of long duration of cigarette smoking for subjects with polyps that overexpress *p53* protein relative to those without overexpression was 0.6 (95%CI = 0.2–1.5).

In addition to these main analyses, we conducted sensitivity analyses a more strigent (intensity score strong and at least 25% or more of cells showing evidence of overexpression) as well as a less stringent definition (intensity score of moderate or strong with at least 10% or more of cells showing evidence of overexpression) of *p53* protein positivity. These analyses, reported in Table 4 suggested that the findings for long duration of cigarette smoking were robust to changes in the definition of *p53* positivity whereas the alcohol findings were not robust to changes in the definition. Specifically, the association seen with heavy alcohol consumption was only seen using our *a priori* definition for positivity and the more stringent defi-

nition of positivity, it was not seen using the more liberal definition of positivity.

Discussion

The *p53* tumor suppressor gene is a commonly mutated gene in colorectal cancer [3]. While the effect of *p53* mutations on colorectal cancer prognosis has been heavily studied, less is known about how epidemiologic risk factors such as alcohol and tobacco consumption relate to *p53* status. In many populations, colorectal adenomas are common [16,24] but most adenomas will not advance to cancer. Understanding risk factors for early colorectal neoplasia is therefore is crucial to colon cancer prevention [18].

p53 status and polyp site

In this study, adenomatous polyps overexpressing *p53* protein (with and without CIS and IM) were more likely to be found in the rectum and distal colon than in the proximal colon ($p < 0.01$). This findings agrees with a study by Diez and colleagues of invasive colorectal cancer which also found p53 overexpression more frequently in distal than proximal tumors (58.5% versus 41.7%, $p = 0.03$)[25].

p53 status and cigarette smoking

We also found that long duration of cigarette smoking was related to adenomatous polyps (with and without CIS and IM) that did not overexpress *p53* protein (OR = 1.8, 95% CI = 1.1–2.9) but not those polyps that did overexpress *p53* protein. This association persisted when only examining CIS and IM cases compared to control and was robust to different definitions of *p53* positivity. This finding also closely matches that of Freedman and colleagues in their case-control study of invasive colorectal cancer: OR = 1.84 (95% CI, 1.00–3.37) with heavy cigarette smoking (40+ pack years) for *p53* negative colorectal cancer and no association with *p53* positive colorectal cancer as determined by protein overexpression [26]. Our findings also lend support to recent findings by Slattery and colleagues on the association between cigarette smoking and microsatellite instability (MSI) positive invasive colon cancers (OR = 1.6, 95%CI = 1.0–2.5 for men, OR = 2.2, 95%CI = 1.4–3.5 for women) but not MSI negative cancers[27]. MSI and *p53* are inversely associated suggesting two molecularly distinct forms of colorectal cancer, MSI-positive and MSI-negative, or, as referred to by Laurent-Puig, LOH (loss of heterozygosity)-positive, characterized by K-ras and *p53* mutations, and MSI-positive colorectal cancers [28,29].

The association between cigarette smoking and *p53*-negative tumors is interesting in light of the relationship between smoking, *p53* mutation, and other tumor sites [30]. People with *p53* mutations who smoke may be more likely to get another cancer, as *p53* mutations are associated with a number of tobacco-related tumors including lung, head and neck, and bladder cancers [30]. Most of the *p53* mutations in colonic tissue, however, are endogenous [4,31] (i.e., spontaneous transitions at the CpG sites); endogenous mutations are not thought to be associated with exogenous environmental factors like cigarette smoking [4].

p53 status and alcohol intake

We found alcohol intake, specifically heavy beer consumption, to be more associated with polyps that overexpress *p53* than those that do not (OR = 4.0, 95% CI = 1.3–12.0 for *p53* protein overexpression relative to polyp-free controls versus OR = 1.6, 95%CI = 0.7–3.7). A similar association was found when restricting the analyses to just the advanced adenomas (CIS and IM). Sensitivity analyses, however, revealed that this finding was limited to our *a priori* definition of *p53* positivity as well as a more strigent definition of positivity but not to a less strigent definition of positivity. Our finding with heavy alcohol consumption agrees with those of Fredrickson and colleagues who reported an increased association of *p53*-positive (as determined by protein overexpression) invasive colorectal cancer with alcohol use (OR = 3.4, 95%CI = 1.1–10)[32].

The association between *p53* protein overexpression and alcohol intake in the colon is interesting in light of the fact, as previously mentioned, that most *p53* mutations in the colon are transitions rather than transversions. In contrast, other tumors such as those of the aerodigestive tract, which are highly associated with smoking and alcohol intake [33] have a higher prevalence of transversions [34]. This suggests the possibility of a different mechanism for alcohol in the colon than aerodigestive tract tumors.

Strengths and Limitations

Our main findings that 1) polyps overexpressing *p53* protein are more likely to be located in the distal colon and rectum, 2) long-term cigarette smoking is more likely to be related to early colorectal tumors that do not overexpress *p53* protein, and 3) alcohol consumption, specifically beer consumption, is more likely to be associated with early colorectal tumors that overexpress *p53* protein, all agree with the literature on invasive colorectal cancer. This suggests that underlying mechanisms that may explain associations between epidemiologic risk factors and *p53* status are likely involved in early colorectal neoplasia as well.

Our study benefited from uniform pathologic review and classification of all lesions, reducing the degree of measurement error in the classification of lesions [35]. *p53* status was also assessed in a blinded fashion. We were able

to obtain paraffin-embedded tissue blocks for 75% of the IM cases and 80% of the CIS cases. Risk factor data did not differ between those with tumor blocks available and those without (data not shown). Uniform classification, blinded assessment, and lack of selection bias in tumor block availability are strengths of this study.

Despite the agreement with the invasive cancer literature and the other study strengths, there are some limitations that warrant discussion. Tests of statistical heterogeneity were not significant. Thus, even though findings between *p53* status and the risk factors were significant relative to polyp-free controls, tests comparing the odds ratios between subjects with *p53* protein overexpression and those without were not statistically significant. Measurement error of *p53* protein overexpression likely hampered the ability to detect heterogeneity [36]. Third, we were limited by a small sample size to fully model differences stratified by *p53* status and case group (adenomatous polyp, CIS, and IM). However, univariate assessment between risk factors and *p53* status stratified by case group suggested similar associations irrespective of case group. Restriction of our final model to just cases with CIS and IM also did not alter any of our conclusions. As well, reliability analyses suggest that CIS and IM cases should be combined into a case group of "advanced adenomas" given the difficulty in reliably classifying these lesions [35].

Conclusion

In sum, we found similar associations between *p53* status and polyp site, cigarette use, and alcohol consumption reported in several studies of invasive colorectal cancer.

Specifically, *p53* protein overexpression, which has been associated with a worse overall survival after cancer diagnosis, is more likely to be found in polyps in the distal colon and rectum, more likely to be associated with alcohol intake, and less likely to be associated with cigarette smoking. We did not find any association between *p53* status and other risk factors for colorectal neoplasia such as body size and physical activity. Additional studies of early colorectal neoplasia that investigate associations between epidemiologic risk factors and markers of genetic changes are needed to understand progression to invasive cancer from a relatively common precursor lesion. However, this study lends itself to the view that some colorectal risk factors may share the same association with respect to specific genotypic changes in precursor lesions and invasive cancer.

Competing Interests

None declared.

Authors' contributions

MBT designed the study and conducted the statistical analyses. HH and MM conducted the pathology review and *p53* assays and NH contributed his pathology expertise. AIN and JW conducted and contributed to the overall parent study. All authors read and approved the final manuscript.

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