Tobacco, alcohol, and \( p53 \) overexpression in early colorectal neoplasia

Mary Beth Terry*1,2, Alfred I Neugut1,2,3, Mahesh Mansukhani4, Jerome Waye5, Noam Harpaz6 and Hanina Hibshoosh4

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 IgG2a [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°C for 30 minutes. Afterwards, the slides are deparaffinized, hydrated, placed 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.
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 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.

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 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 ± 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 (6.3%)                               |          |
| 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 (µ ± σ) | 25.3 (11.3)                             | 29.6 (14.5)                            | 0.17     |
| Total years (µ ± σ)  | 29.2 (14.8)                               | 24.6 (13.6)                            | 0.23     |
| Cigs per day (µ ± σ) | 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 (µ ± σ) | 10.5 (15.6)                             | 12.6 (15.8)                            | 0.50     |
| **Other risk factors**|                                        |                                        |          |
| Body Mass Index (kg/m²) (µ ± σ) | 25.2 (4.3)                             | 25.6 (5.3)                             | 0.64     |
| Leisure Physical Activity Hours/week (µ ± σ) | 0.6 (2.6)                             | 0.9 (1.9)                              | 0.69     |
| Daily Fiber Intake Grams/day (µ ± σ) | 16.3 (6.9)                             | 16.1 (6.6)                             | 0.86     |
| Daily Fat Intake Grams/day (µ ± σ) | 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.

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.
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/m²)        |                               |                            |
| < 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)        |
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 def-
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 finding 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 stringent definition of positivity but not to a less stringent 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. AJN and JW conducted and contributed to the overall parent study. All authors read and approved the final manuscript.

**References**

1. Fearon ER and Vogelstein B: A genetic model for colorectal tumorigenesis. Cell 1990, 61:759-767.
2. Cho KR and Vogelstein B: Genetic alterations in the adenoma-carcinoma sequence. Cancer 1992, 70:1727-1731.
3. Fearon ER: Molecular genetic studies of the adenoma-carcinoma sequence. Adv Int Med 1994, 39:123-147.
4. Hamilton SR: The molecular genetics of colorectal neoplasia. Gastroenterology 1993, 105:3-7.
5. Ilyas M and Talbot IC: p53 expression in ulcerative colitis: a longitudinal study. Gut 1995, 37:802-804.
6. Pignatelli M, Stamp GW, Kafiri G, Lane D and Bodmer WF: Overexpression of p53 nuclear oncoprotein in colorectal adenomas. Int J Cancer 1992, 50:683-688.
7. Kikuchi-Yanoshita R, Konishi M, Ito S, Seki M, Tanaka K, Maeda Y, Iino H, Fukayama M, Koike M and Mori T: Genetic changes of both p53 alleles associated with the conversion from colorectal adenoma to early carcinoma in familial adenomatous polyposis and non-familial adenomatous polyposis patients. Cancer Research 1992, 52:3965-3971.
8. Hamilton SR: Molecular genetics of colorectal cancer. Cancer 1992, 70:1216-1221.
9. Taylor CR, O’Grady J, Chwajun B, Young L, Imam SA and Cote RJ: Epidemiology of colorectal adenomatous polyposis. Cancer Epidemiol Biomarkers Prev 1993, 2:159-176.
10. Hoff G and Vatn MH: Colonic adenoma: natural history. Dig Dis Sci 1991, 36:1-69.
11. Terry MB, Neugut AI, Bostick RM, Sandler RS, Haile RW, Jacobson JS, Fenoglio-Preiser CM and Potter JD: Risk factors for advanced colorectal adenomas: a pooled analysis. Cancer Epidemiol Biomarkers Prev 2002, 11:622-629.
12. Neugut AI, W.C. Lee, Garbowski GC, Wake JD, Forde KA, Treat MR and Fenoglio-Preiser C: Obesity and colorectal adenomatous polyposis. J Natl Cancer Inst 1991, 83:359-361.
13. Terry MB and Neugut AI: Smoking and the adenoma-carcinoma sequence. Am J Epidemiol 1998, 147:903-910.
14. Taylor CR, Shi SR, Chwajun B, Young L, Imam SA and Cote RJ: Strategies for improving the immunohistochemical staining of various intranuclear prognostic markers in formalin-paraffin sections. Human pathology 1994, 25:263-270.
22. Hosmer D.W. Jr, and Lemeshow S.: Applied Logistic Regression New York, Wiley & Sons; 1989:216-238.
23. Rothman KJ and Greenland S.: Modern Epidemiology. Philadelphia, Lipincott-Raven; 1998:329-342.
24. Peipins LA and Sandler RS: Epidemiology of colorectal adenomas. Epidemiol Rev 1994, 16: 273-297.
25. Diez M, Medrano M, Muguerza JM, Ramos P, Hernandez P, Villela R, Martin A, Nogueiras F, Ruiz A and Granell J: Influence of tumor localization on the prognostic value of p53 protein in colorectal adenocarcinomas. Anticancer Res 2000, 20:3907-3912.
26. Freedman AN, Michalek AM, Marshall JR, Metcalf CJ, Petrelli NJ, Zhang ZF, Black JD, Satchidanand S and Asirwatham JE. The relationship between smoking exposure and p53 overexpression in colorectal cancer. Br J Cancer 1996, 73:902-908.
27. Slattery ML, Curtin K, Anderson K, Ma KN, Ballard L, Edwards S, Schaffer D, Potter J, Leppert M and Samowitz WS: Associations between cigarette smoking, lifestyle factors, and microsatellite instability in colon tumors. J Natl Cancer Inst 2000, 92:1831-1836.
28. Laurent-Puig P, Blons H and Cugnenc PH: Sequence of molecular genetic events in colorectal tumorigenesis. Eur J Cancer Prev. 1999, 9:539-47.
29. Neugut AI and Terry MB: Cigarette smoking and microsatellite instability: causal pathway or marker-defined subset of colon tumors? J Natl Cancer Inst. 2000, 92:1791-1793.
30. Harris CC: 1995 Deichmann Lecture - p53 tumor suppressor gene: at the crossroads of molecular carcinogenesis, molecular epidemiology and cancer risk assessment. Toxicology Letters 1995, 82-83:1-7.
31. Hollstein M, Sidransky D, Vogelstein B and Harris CC: p53 mutations in human cancers. Science 1991, 253 (5015):49-53.
32. Fredriksson M, Axelsson O, Sun XF, Arbman G, Nilsson E, Nordenskjold B, Sipdahl R and Soderkvist P: A pilot study on risk factors and p53 gene expression in colorectal cancer. Br J Cancer 1996, 73:1428-1430.
33. Kato H, Yoshikawa M, Miyazaki T, Nakajima M, Fukai Y, Tajima K, Masuda N, Tsukada K, Fukuda T, Nakajima T and Kuvano H: Expression of p53 protein related to smoking and alcoholic beverage drinking habits in patients with esophageal cancers. Cancer Lett 2001, 167:65-72.
34. Olshan AF, Weissler MC, Pei H and Conway K: p53 mutations in head and neck cancer: new data and evaluation of mutational spectra. Cancer Epidemiol Biomarkers Prev 1997, 6:499-504.
35. Terry MB, Neugut AI, Bostick RM, Potter JD, Haile RW and Fenoglio-Preiser CM: Reliability in the classification of advanced colorectal adenomas. Cancer Epidemiol Biomarkers Prev 2002, 11:660-663.
36. Terry MB, Gammon MD, Ng-Mak D and Thompson WD: Re: "p53 protein overexpression in relation to risk factors for breast cancer". Am J Epidemiol 1998, 147:511-512.

Pre-publication history
The pre-publication history for this paper can be accessed here:

http://www.biomedcentral.com/1471-2407/3/29/prepub