Assessing Tumor Mutations to Gain Insight into Base Excision Repair Sequence Polymorphisms and Smoking in Colon Cancer

Karen Curtin,1 Wade S. Samowitz,2 Roger K. Wolff,1 Cornelia M. Ulrich,3 Bette J. Caan,4 John D. Potter,3 and Martha L. Slattery1,5

Departments of1Internal Medicine and2Pathology, University of Utah Health Sciences Center, Salt Lake City, Utah;3Cancer Prevention Program, Fred Hutchinson Cancer Research Center, Seattle, Washington;4Division of Research, Kaiser Permanente Medical Care Program, Oakland, California; and5Department of Oncology, Tom Baker Cancer Center, University of Calgary, Alberta, Canada

Abstract

DNA repair enzymes function in major pathways to reverse DNA damage, including base excision repair (BER). Missense polymorphisms in BER repair genes may contribute to differences in DNA repair capacity, specific mutations, and susceptibility to cancer in the presence of exposure to carcinogens such as cigarette smoking. In a study of 1,604 incident colon cancer cases and 1,969 matched population-based controls genotyped for BER variants OGG1 (S326C) and XRCC1 (R194W, R280H, and R399Q), we found no associations with colon cancer overall. However, a 2-fold increased risk of BRAF V600E tumor mutation was observed in current and former cigarette smokers homozygous for the OGG1 polymorphism (odds ratio, 2.2; 95% confidence interval, 1.02-4.9, recessive model); similar associations were not observed for microsatellite instability, CpG island methylator phenotype, KRAS2 mutations, or TP53 mutations. The XRCC1 R194W polymorphism was associated with a modest increased risk of TP53 tumor mutations in those who regularly smoked cigarettes (odds ratio, 1.4; 95% confidence interval, 1.02-1.9). These findings point to the importance of studying tumor mutations when examining DNA repair polymorphisms and cigarette smoke exposure to identify potentially relevant associations with colorectal cancer. (Cancer Epidemiol Biomarkers Prev 2009;18(12):3384–8)

Introduction

An extensive system of DNA repair enzymes function in major pathways to reverse DNA damage to DNA including base excision repair (BER), double-strand break repair, and nucleotide excision repair. Missense polymorphisms in DNA repair genes may contribute to differences in DNA repair capacity in these pathways and influence susceptibility to cancer, particularly in the presence of exposure to carcinogens such as cigarette smoke (1).

In BER, lesions consisting of modified DNA bases or single-strand breaks are repaired by one or more nucleotides through different protein networks (2). OGG1-initiated BER oxidative DNA damage acts to remove 8-oxoguanine from DNA and restore the original sequence (3). Reduced activity of oxoguanine DNA glycosylase (OGG) has been identified as a risk factor for lung, and head and neck cancers (4). A missense polymorphism in codon 326 of the OGG1 gene (C>G change at position 1245) results in a cysteine substitution for serine (S326C, dbSNP no. rs1052133). As the colon may be subject to exposure to oxygen free radicals, reduced activity of OGG1 may be a risk factor in colon carcinogenesis. However, a limited number of studies of S326C and colon cancer have been conducted (5, 6). Another protein, XRCC1, is critical in the BER pathway, interacting with several BER enzymes to modify and stabilize their activity (2). Common XRCC1 sequence variants, R194W (C>T; rs1799782), R280H (G>A; rs25489), and R399Q (GA; rs25487), have been previously implicated in risk of colorectal cancers (CRCs) and adenomas, although smoking did not seem to modify associations in previous reports with limited sample sizes that may have been too small to detect associations (7).

DNA repair polymorphisms and mediating effects of environmental exposures have been well studied in sporadic CRC; few reports have examined the association of common variants in these pathways with somatic tumor alterations. In previous reports, we examined DNA mismatch repair gene polymorphisms in MLH1 and MSH6 and risk of genetic and epigenetic changes in colon tumors with risk factors including tobacco smoke (8, 9). We have also presented findings to support that smoking
can preferentially predispose to transversion mutations in TP53 and KRAS2, and the BRAF V600E mutation, as well as microsatellite instability (MSI) and a CpG island methylator phenotype (CIMP) in CRC tumors (10-14). This investigation is, to our knowledge, the first large population-based case-control study in which both BER coding polymorphisms of incident colon cancers characterized for tumor marker status, including MSI, CIMP, BRAF, KRAS2, and TP53 mutations, and cigarette smoking exposure were collected.

Materials and Methods

Study Population. Participants in the study were from the Kaiser Permanente Medical Care Program of Northern California, the Twin Cities Metropolitan area in Minnesota, or an eight-county area in Utah (Davis, Salt Lake, Utah, Weber, Wasatch, Tooele, Morgan, and Summit counties). All eligible incident cases with a first primary colon tumor within these defined populations were identified between October 1, 1991 and September 30, 1994 and recruited for the study. Cases with a previous colorectal tumor, familial adenomatous polyposis, ulcerative colitis, or Crohn’s disease documented on the pathology report were not eligible for the study. In addition to these criteria, participants were between 30 and 79 y of age at time of diagnosis, English speaking, and mentally competent to complete the interview. Using the same eligibility criteria, controls were frequency matched to cases by sex and by 5-year age cohort. At Kaiser Permanente Medical Care Program of Northern California, controls were randomly selected from membership lists. At Minnesota and Utah, controls younger than 65 y were randomly selected from random-digit dialing or driver’s license lists and controls 65 y and older were randomly selected from social security lists.

Institutional review board approval was obtained from all study centers. A total of 1,604 colon cancer cases (898 men and 706 women) and 1,969 controls (1,040 men and 929 women) with genotype data were included in the study. The race/ethnicity of the study population, self-reported at the time of interview, was primarily White, non-Hispanic (91% of cases and 93% of controls). The remainder were Hispanic (4% of cases and 4% of controls) or African-American (9% of cases and 9% of controls). The median age of cases at diagnosis and controls at selection was 64 y. Of all cases asked to participate, 75.6% cooperated; of controls selected, 63.7% participated as previously described (15).

Data Collection. Data were collected for cases and controls by trained and certified interviewers for a calendar-year reference period 2 y before year of diagnosis for cases or selection for controls; rigorous quality control methods were used (16). Anthropometrics and a detailed diet and life-style history were collected. Long-term vigorous physical activity data were collected using a detailed physical activity questionnaire (15). Participants were asked to report number of years they smoked, the age they stopped smoking (if former smoker), and usual number of cigarettes smoked per day while smoking regularly. Of cases, 14% were current and 45% were former smo-

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Genotype</th>
<th>Control</th>
<th>Case</th>
<th>MSI+</th>
<th>Case</th>
<th>CIMP high</th>
<th>Case</th>
<th>BRAF V600E</th>
<th>Case</th>
<th>KRAS2 mutation</th>
<th>Case</th>
<th>TP53 mutation</th>
<th>Case</th>
</tr>
</thead>
<tbody>
<tr>
<td>OGG1</td>
<td>S126C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>1,472</td>
<td>1,172</td>
<td>1.0 (Reference)</td>
<td>120</td>
<td>1.0 (Reference)</td>
<td>184</td>
<td>1.0 (Reference)</td>
<td>60</td>
<td>1.0 (Reference)</td>
<td>201</td>
<td>1.0 (Reference)</td>
<td>310</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>1,172</td>
<td>918</td>
<td>1.0 (Reference)</td>
<td>120</td>
<td>1.0 (Reference)</td>
<td>184</td>
<td>1.0 (Reference)</td>
<td>60</td>
<td>1.0 (Reference)</td>
<td>201</td>
<td>1.0 (Reference)</td>
<td>310</td>
</tr>
<tr>
<td></td>
<td>G/G</td>
<td>570</td>
<td>418</td>
<td>1.0 (Reference)</td>
<td>55</td>
<td>1.0 (Reference)</td>
<td>72</td>
<td>1.0 (Reference)</td>
<td>14</td>
<td>1.0 (Reference)</td>
<td>37</td>
<td>1.0 (Reference)</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>G/C</td>
<td>694</td>
<td>560</td>
<td>1.0 (Reference)</td>
<td>65</td>
<td>1.0 (Reference)</td>
<td>92</td>
<td>1.0 (Reference)</td>
<td>28</td>
<td>1.0 (Reference)</td>
<td>43</td>
<td>1.0 (Reference)</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>C/C</td>
<td>210</td>
<td>160</td>
<td>1.0 (Reference)</td>
<td>17</td>
<td>1.0 (Reference)</td>
<td>27</td>
<td>1.0 (Reference)</td>
<td>9</td>
<td>1.0 (Reference)</td>
<td>19</td>
<td>1.0 (Reference)</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>1,472</td>
<td>1,172</td>
<td>1.0 (Reference)</td>
<td>120</td>
<td>1.0 (Reference)</td>
<td>184</td>
<td>1.0 (Reference)</td>
<td>60</td>
<td>1.0 (Reference)</td>
<td>201</td>
<td>1.0 (Reference)</td>
<td>310</td>
</tr>
</tbody>
</table>

Table 1. Association of BER sequence polymorphisms and colon tumors

* ICD-O 2nd edition codes 18.0 and 18.2 through 18.9, determined by the Surveillance Epidemiology and End Results Cancer Registries in Utah and Northern California.
Colon Tumor Mutations, BER Polymorphisms, and Smoking

Table 2. Association of BER sequence polymorphisms and colon tumors in current or former smokers

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Case</th>
<th>All cases</th>
<th>Case</th>
<th>MSI+</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>n°</td>
<td>OR (95% CI)</td>
<td>n</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>OGG1</td>
<td>S326C</td>
<td>CC</td>
<td>620</td>
<td>556</td>
<td>1.0 (Reference)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CG</td>
<td>372</td>
<td>321</td>
<td>1.0 (0.8, 1.2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GG</td>
<td>45</td>
<td>54</td>
<td>1.4 (0.9, 2.2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P_trend</td>
<td>0.44</td>
<td>1.5 (1.0, 2.2)</td>
<td>0.63</td>
</tr>
<tr>
<td>XRCC1</td>
<td>R194W</td>
<td>CC</td>
<td>925</td>
<td>804</td>
<td>1.0 (Reference)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CG</td>
<td>111</td>
<td>127</td>
<td>1.3 (1.0-1.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GG</td>
<td>946</td>
<td>847</td>
<td>1.0 (Reference)</td>
</tr>
<tr>
<td>XRCC1</td>
<td>R280H</td>
<td>GA/AA</td>
<td>90</td>
<td>84</td>
<td>1.1 (0.8-1.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GA</td>
<td>424</td>
<td>393</td>
<td>1.0 (Reference)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AA</td>
<td>477</td>
<td>424</td>
<td>1.0 (0.8-1.2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P_trend</td>
<td>0.59</td>
<td>0.59</td>
<td>0.68</td>
</tr>
</tbody>
</table>

NOTE: Adjusted for age, sex, race, center, energy, body mass index, activity, long-term alcohol use, recent NSAIDs, and family history.

*Includes 689 cases with tumor marker data.

†Recessive model.

TABLE 2. Association of BER sequence polymorphisms and colon tumors in current or former smokers

Genotyping. Genomic DNA was extracted from peripheral WBC collected from blood drawn at the time of the study interview using the Puregene kit (Genta Systems). From available DNA, genotyping was successfully conducted for 1,604 cases and 1,996 controls. Missing genotyping data were due to lack of amplification or ambiguous results (<0.5%). The OGG1 S326C and polymorphisms in XRCC1 (R194W, R280H, and R399Q) were detected by allelic discrimination using TaqMan assays on a 7900HT sequence detection system (Applied Biosystems). Positive controls for all the genotypes as well as four negative controls were included in each plate. For quality control purposes, genotyping for 94 randomly selected samples was repeated. There were no discrepancies. All genotypes were in Hardy-Weinberg equilibrium. Minor allele frequencies in cases and controls, by race/ethnicity, are shown in Supplementary Table S1 (online). As part of a larger study, XRCC3 T241M, ERCC2 (D312N and K751Q), ERCC5 D1104H, and MGMT (L84F and I143V) were also genotyped using these methods as previously described (17).

Tumor Analysis. Tumor DNA was obtained from paraffin-embedded tissue. Tumors were characterized by their genetic profile that included TP53 sequence data for mutation hotspots of exons 5 through 8; sequence data for KRA52 codons 12 and 13; five CpG Island markers that are considered to be markers of CIMP (18, 19), methylated in tumors MINT1, MINT2, and MINT31, CDKN2A (p16), and MLH1; the V600E BRAF mutation; and MSI status determined by BAT26 and TGFβRII. For MSI, the majority of tumors were classified as MSI positive (MSI+), small subset of tumors that had neither BAT26 nor TGFβRII results were classified using a panel of 10 tetranucleotide repeats. These methods have been previously described in detail (10, 20). Of 1,604 genotyped cases, tumor markers were assessed in 1,210 colon cancers.

Statistical Analysis. All statistical analyses were done using SAS version 9.2 (SAS Institute). Tumors were defined by specific alterations detected; MSI+, BRAF V600E, any KRAS2 mutation, any TP53 mutation, or CIMP high defined as methylation of two or more of five markers. Additionally, KRAS2 or TP53 status was further defined by the presence of any transition or any transversion mutation. Population-based controls were used to assess associations for the population overall while examining multiple outcomes defined by tumor status. A multiple logistic regression model was used to compare all interviewed cases, regardless of whether or not tumor tissue was obtained, to controls. To compare specific types of mutations to controls while adjusting for the other tumor mutations simultaneously, a generalized estimating equation with a multinomial outcome was used as case subjects could contribute from one to five outcome observations depending upon how many tumor alterations or mutations (MSI+, CIMP high, BRAF, KRAS2, TP53) an individual had (21). The generalized estimating equation accounts for correlation introduced by including subjects multiple times and was implemented using the GENMOD procedure as described by Kuss and McLerran (22). All models were adjusted for sex, age at diagnosis or selection, study center, race/ethnicity, total energy (kcal), body mass index in kg/m², long-term vigorous physical activity, long-term alcohol use, cigarette smoking (never, current, or former smoker) in combined analyses not stratified by smoking, recent NSAID use, and family history of CRC in first-degree relatives.

We assessed odds ratios (OR) and 95% confidence intervals (95% CI) in logistic regression models (colorectal cancer overall) and in generalized estimating equation models for tumor mutation outcomes. Analyses were stratified by cigarette smoking status dichotomized as never smoked and current/former smoker. P for trend was assessed over using ordered categories of variables and comparing the likelihood ratio of a model with the variable to the likelihood ratio of a model without the variable using a χ² test with one degree of freedom. P for interaction was determined by comparing a full model including an ordinal multiplicative interaction term to a reduced model without an interaction term, using a likelihood ratio test.
## Results

Associations of BER pathway missense polymorphisms in OGG1 and XRCC1 are shown in Table 1 for colon cancer overall and by tumor alteration or mutation. The largest effect size, a nonstatistically significant 1.7-fold increased risk of BRAF V600E mutation, was observed in homozygous carriers of the OGG1 S326C variant. Coding polymorphisms in other DNA repair pathways that were examined, double-strand break repair (XRCC3 T241M), nucleotide excision repair (ERCC2 D312N and K751Q, ERCC5 D1104H), and direct repair gene MGMT (L84F and I1143V), were generally not associated with colon cancer or colon tumor subtypes compared with controls, and thus, data are not shown; however, a supplementary table of results is available online (Supplementary Table S2). To further examine associations of BER sequence variants, we examined associations among those who ever smoked cigarettes (Table 2, case-control comparison). In those who currently or formerly smoked cigarettes on a regular basis (53% of controls and 57% of cases with tumor marker data), homozygous carriers of the OGG1 variant were twice as likely to harbor a BRAF tumor mutation compared with individuals with a genotype containing 0 or 1 variant allele. As heterozygous individuals did not exhibit any increased risk of BRAF, a recessive model may be appropriate, as shown; however, the number of cases homozygous for S326C is small. BRAF mutation in colon cancer often occurs in conjunction with MSI or CIMP; however, OGG1 S326C did not confer an increased risk in these tumor outcomes (Table 2). An interaction term only approached statistical significance in a case-control comparison of BRAF-mutated and nonmutated tumors (P = 0.08) and was not significant in a case-control comparison (P = 0.41; data not shown). As ever smokers comprised over two thirds of cases with a BRAF tumor mutation (68%), the numbers may be small to detect an interaction due to relatively few nonsmokers in the analysis who exhibited this alteration.

Missense polymorphisms in XRCC1 were generally not associated with risk of colon cancer; however, carriage of one or two variant R194W alleles was associated with a modest 1.4-fold increased risk of a TP53 mutation in smokers (Table 2), with the association signal observed more prominently in transversion mutations (OR, 1.7; 95% CI, 0.9-3.2; data not shown). As the R194W and R280H minor alleles are relatively low common (frequencies of 0.06 and 0.05, respectively), a dominant model is shown. XRCC1 haplotypes or combined genotype combinations across R194W, R280H, and R399Q were not associated with colon cancer or tumor subtype.

## Discussion

Similar to other reports, we found little evidence to support a strong involvement of common nonsynonymous variants in the BER, double-strand break repair, nucleotide excision repair, and direct DNA repair pathways in overall colon cancer etiology. However, within tumor subphenotypes, there was a suggestive association of OGG1 in the BER pathway and BRAF colon tumor mutation in those who ever smoked cigarettes, with current or former smokers comprising the majority of BRAF-mutated cases. Although some studies have reported no difference in 8-OHdG levels or repair activity between S326C genotypes in functional assays, the ability to suppress spontaneous mutagenesis has been shown to be significantly lower for OGG1 protein encoded by the variant C allele than for the S allele in lung cancer cell lines (4, 5). Smoking in combination with lower OGG activity has been associated with higher cancer risk for lung and esophageal cancer. Among smokers, homozygous carriers of the S326C allele who putatively have a decreased ability to cope with oxidative DNA damage are more susceptible to lung cancer than smokers who carry no or one copy of the variant (5).

Interestingly, OGG1 S326C was not associated with MSI+ or CIMP-high colon cancers overall or in current and former smokers in our study; BRAF V600E mutations are less common in colon cancers (9%) than either MSI+ or CIMP-high tumors (14% and 27%, respectively; ref. 23). A majority of BRAF-mutated tumors exhibit CIMP (and additionally, MSI) and are thought to be a less frequent alternate event in the CIMP-high pathway (24). As we used generalized estimating equations to model multiple tumor outcomes in which tumors can exhibit more than one somatic alteration event to account for correlation introduced by including cases multiple times, the estimate of increased risk of BRAF colon cancer in homozygous S326C carriers who smoked seems to be independent of whether or not their tumor also exhibited a CIMP-high phenotype. Smoking has been previously associated with hyperplastic polyps (25), and BRAF mutations seem to be involved in the formation of hyperplastic polyps, which may be the precursor for sporadic MSI+ and CIMP-high tumors (26).

### Table 2. Association of BER sequence polymorphisms and colon tumors in current or former smokers (Cont’d)

<table>
<thead>
<tr>
<th>Case</th>
<th>CIMP high</th>
<th>Case</th>
<th>BRAF mutation</th>
<th>Case</th>
<th>KRAS2 mutation</th>
<th>Case</th>
<th>TP53 mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>OR (95% CI)</td>
<td>n</td>
<td>OR (95% CI)</td>
<td>n</td>
<td>OR (95% CI)</td>
<td>n</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>114</td>
<td>1.0 (Reference)</td>
<td>40</td>
<td>1.0 (Reference)</td>
<td>116</td>
<td>1.0 (Reference)</td>
<td>188</td>
<td>1.0 (Reference)</td>
</tr>
<tr>
<td>50</td>
<td>0.8 (0.6-1.1)</td>
<td>8</td>
<td>0.8 (0.5-1.4)</td>
<td>69</td>
<td>1.1 (0.8-1.4)</td>
<td>116</td>
<td>1.1 (0.9-1.4)</td>
</tr>
<tr>
<td>114</td>
<td>1.0 (Reference)</td>
<td>0.36</td>
<td>2.1 (0.9-4.7)</td>
<td>10</td>
<td>1.1 (0.6-2.2)</td>
<td>14</td>
<td>1.0 (0.5-1.7)</td>
</tr>
<tr>
<td>72</td>
<td>0.9 (0.7-1.2)</td>
<td>26</td>
<td>0.25 (0.1-0.9)</td>
<td>19</td>
<td>0.7 (0.5-1.2)</td>
<td>40</td>
<td>1.1 (0.7-1.5)</td>
</tr>
<tr>
<td>145</td>
<td>1.0 (Reference)</td>
<td>0.90</td>
<td>0.43</td>
<td>8</td>
<td>0.9 (0.5-1.8)</td>
<td>6</td>
<td>1.2 (0.5-2.5)</td>
</tr>
<tr>
<td>74</td>
<td>1.0 (Reference)</td>
<td>33</td>
<td>1.0</td>
<td>6</td>
<td>1.2 (0.5-2.5)</td>
<td>8</td>
<td>1.0 (Reference)</td>
</tr>
<tr>
<td>72</td>
<td>1.0 (Reference)</td>
<td>33</td>
<td>1.0</td>
<td>6</td>
<td>1.2 (0.5-2.5)</td>
<td>8</td>
<td>1.0 (Reference)</td>
</tr>
<tr>
<td>145</td>
<td>1.0 (Reference)</td>
<td>0.90</td>
<td>0.43</td>
<td>8</td>
<td>0.9 (0.5-1.8)</td>
<td>6</td>
<td>1.2 (0.5-2.5)</td>
</tr>
</tbody>
</table>


Downloaded from cebp.aacrjournals.org on June 13, 2021. © 2009 American Association for Cancer Research.
We previously reported smoking in association with both CIMP-high and BRAF mutations in colon cancers (13). More than two thirds of cases (68%) with a BRAF mutation were current or former smokers. Our finding that smoking may interact with OGG1 S326C to increase risk is suggestive and must be interpreted with caution. Although our case-control study of colon tumors was large, numbers to detect a statistically significant interaction with both OGG1 genotype and smoking in BRAF mutated tumors were small. Our results indicate the possibility that decreased enzyme activity from a non-synonymous variant in the BER pathway may be involved in predisposing colon tumors to BRAF V600E in current and former regular users of tobacco.

Common polymorphisms in BER or other DNA repair pathways have been examined in colorectal tumor subtypes in a very limited number or reports. Park et al. (6) reported that the S326C polymorphism was not associated with tumor location or MSI status in CRC patients, and Sliwinski et al. (27) did not find a correlation between this variant and CRC occurrence or progression. In a study of 125 cases and 247 matched controls, Kim et al. (5) previously reported the homozgyous variant S326C genotype was associated with colon cancer in smokers. Our findings lend further support to the hypothesis that risk of colon tumors may be influenced by OGG1 genotype in the presence of environmental exposures such as smoking (4); however, the increased risk was observed in tumors with positive BRAF V600E mutation status. We recognize that in our hypothesis-based investigation, the associations we observed may be due to chance as a number of comparisons were made; thus, our findings should be interpreted with caution and replication in other studies is warranted.

This investigation points out that putative functional risk alleles and cigarette smoke exposure in sporadic CRC needs to be studied in tumors assessed for mutation risk alleles and cigarette smoke exposure in sporadic CRC cancers. We previously reported smoking in association with BRAF and CpG island methylator phenotype in colon cancer (17). More than two thirds of cases (68%) with a V600E mutation status. We recognize that the increased risk was observed in tumors with positive BRAF V600E mutation status. We recognize that in our hypothesis-based investigation, the associations we observed may be due to chance as a number of comparisons were made; thus, our findings should be interpreted with caution and replication in other studies is warranted.

This investigation points out that putative functional risk alleles and cigarette smoke exposure in sporadic CRC needs to be studied in tumors assessed for mutation risk alleles and environmental exposures such as smoking (4); however, the increased risk was observed in tumors with positive BRAF V600E mutation status. We recognize that in our hypothesis-based investigation, the associations we observed may be due to chance as a number of comparisons were made; thus, our findings should be interpreted with caution and replication in other studies is warranted. This investigation points out that putative functional risk alleles and cigarette smoke exposure in sporadic CRC needs to be studied in tumors assessed for mutation risk alleles and environmental exposures such as smoking (4); however, the increased risk was observed in tumors with positive BRAF V600E mutation status. We recognize that in our hypothesis-based investigation, the associations we observed may be due to chance as a number of comparisons were made; thus, our findings should be interpreted with caution and replication in other studies is warranted. This investigation points out that putative functional risk alleles and cigarette smoke exposure in sporadic CRC needs to be studied in tumors assessed for mutation risk alleles and environmental exposures such as smoking (4); however, the increased risk was observed in tumors with positive BRAF V600E mutation status. We recognize that in our hypothesis-based investigation, the associations we observed may be due to chance as a number of comparisons were made; thus, our findings should be interpreted with caution and replication in other studies is warranted.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Acknowledgments
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank Sandra Edwards, Leslie Palmer, and Judy Morse for the data collection and management efforts of this study; Jeanette Bigler for genotyping; and Michael Hoffman and Erica Wolff for technical assistance with this study. The contents of this article are solely the responsibility of the authors and do not necessarily represent the official view of the National Cancer Institute.

References
Assessing Tumor Mutations to Gain Insight into Base Excision Repair Sequence Polymorphisms and Smoking in Colon Cancer


Updated version
Access the most recent version of this article at:
http://cebp.aacrjournals.org/content/18/12/3384

Supplementary Material
Access the most recent supplemental material at:
http://cebp.aacrjournals.org/content/suppl/2009/12/01/18.12.3384.DC1

Cited articles
This article cites 25 articles, 4 of which you can access for free at:
http://cebp.aacrjournals.org/content/18/12/3384.full#ref-list-1

Citing articles
This article has been cited by 4 HighWire-hosted articles. Access the articles at:
http://cebp.aacrjournals.org/content/18/12/3384.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link
http://cebp.aacrjournals.org/content/18/12/3384.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.