XRCC1, *XRCC3*, and *XPD* Polymorphisms as Modifiers of the Effect of Smoking and Alcohol on Colorectal Adenoma Risk

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Abstract

Using a sigmoidoscopy-based case-control study (753 cases, 799 controls) in Los Angeles County, we investigated the potential modifier role in the effect of alcohol and smoking of single-nucleotide polymorphisms (SNP) in three DNA repair genes, *XRCC1* (Arg194Trp and Arg399Gln), *XRCC3* (Thr241Met), and *XPD* (Lys751Gln). We have previously reported an inverse association between the *XRCC1* codon 399 SNP and adenoma risk among these subjects. We now report that subjects with the *XPD* Gln/Gln genotype were inversely associated with adenoma risk [odds ratio (OR), 0.7; 95% confidence interval (95% CI), 0.5-1.0] when compared with subjects with the Lys/Lys and Lys/Gln genotypes combined. This association differed between different ethnic groups (gene × race heterogeneity likelihood ratio test, $P = 0.009$), with a stronger inverse association among Latinos (OR, 0.1; 95% CI, 0.01-0.5) than among non-Latinos (OR, 0.9; 95% CI, 0.-1.3). We found no evidence of an *XRCC3* × smoking or alcohol interaction or an XRCC1 × alcohol interaction. Instead, our data supported an *XRCC1* × smoking interaction ($P = 0.048$). Whereas *XPD* did not modify the effect of smoking, our data suggested an *XPD* × alcohol interaction. Analyses ignoring *XPD* showed no association between alcohol intake and adenoma prevalence; however, among carriers of the codon 751 Gln/Gln genotype, we found a significant positive association (OR, 2.5; 95% CI, 1.2-5.2 for ever drinkers; test of interaction $P = 0.04$). Our data suggest that the effects of smoking and alcohol may vary depending on the genetic background of proteins that participate in the base excision repair and nucleotide excision repair pathways. (Cancer Epidemiol Biomarkers Prev 2006;15(12):2384–90)

Introduction

Epidemiologic studies across different continents have consistently reported that cigarette smoking is a strong adenoma risk factor (1-9). The strength of the associations and the overall consistency of the results across different populations suggest that smoking is likely to be causal. A pooled analysis of various studies showed that smoking is more likely to be a risk factor for adenoma formation rather than adenoma growth or dysplasia (10).

Whereas smoking for <20 years seems to be associated with smaller adenomas, smoking for >20 years tends to associate with larger adenomas (6, 7, 11). In addition, smoking was reported to increase the prevalence of multiple versus single adenomas, as well as adenoma recurrence (6, 7, 12). Altogether, these data support the hypothesis that smoking might be related to a small adenoma to large adenoma sequence. Given the known role of adenomas as precursors of carcinomas (13), these data support the hypothesis that smoking may contribute to the formation of lesions that can later progress to colon carcinomas.

Smoking is a rich source of both reactive oxygen species (14) and chemical carcinogens. Reactive oxygen species, which are present in both the gas-phase and the particulate-phase matter (tar; ref. 14), can initiate lipid peroxidation, oxidize proteins, and cause direct damage to DNA (14). DNA damage includes single- and double-strand breaks, abasic sites, and base adduct formation, such as thymine glycol, 5-hydrozymethyluracil, and 8-hydroxy-2-deoxyguanosine (15). Chemical carcinogens in cigarette smoke (i.e., polycyclic aromatic hydrocarbons, nitrosamines, and arylamines) can induce bulky adducts in crypt cells, thus also contributing to the formation of mutations in the colon. Smoking can also lower levels of antioxidants, such as carotenoids (16, 17), and induce expression of proteins related to colon tumor progression and invasion, such as 5-lipooxygenase, vascular endothelium growth factor, and metalloproteinases (matrix metalloproteinase-2 and matrix metalloproteinase-9), in colon cancer cells (18, 19). Furthermore, smoking may decrease levels of circulating folate and thus oppose the protective effects of folate on adenoma formation (20).

Alcohol intake has inconsistently been reported to be associated with colorectal adenomas. Whereas a majority of epidemiologic studies found a positive association between alcohol intake and adenoma risk (2-4, 8, 9, 20-25), a few studies did not (2, 26-28). A previous study by our group, using phase I subjects only, found a weak association between alcohol intake and adenoma risk (29).

A role for ethanol in adenoma formation is plausible. Alcohol can act as a cocarcinogen by facilitating the absorption of carcinogens, such as those in cigarette smoke (30), and as a carcinogen due to its conversion in the colon lumen to acetaldehyde. The latter can form DNA adducts, such as N^2-ethyl-2′-deoxyguanosine and 1,N^2-propanodeoxyguanosine (31), and interstrand cross-links (31) and can induce oxidative DNA damage such as DNA strand breaks (32). The presence of base adducts such as N^2-ethyl-2′-deoxyguanosine and 1,N^2-propanodeoxyguanosine can induce point mutations in DNA; 1,N^2-propanodeoxyguanosine adducts can also act as topoisomerase II poisons, leading to an increase of unresolved double-strand breaks induced by this enzyme (33). Acetaldehyde can also inhibit the action of O^6-methylguanine-methyltransferase, which is an important enzyme for the removal of

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alkylating damage, such as that induced by cigarette smoking, thus contributing to further accumulation of DNA damage (32). In addition, ethanol has been shown to impair the repair of strand breaks and bulky adducts (34, 35). Ethanol can induce CYP2E1, which has a high redox potential and can induce oxidative stress and lipid peroxidation in the tissues where it is expressed, such as the colon, thus contributing to further DNA damage (32). High alcohol intake can reduce levels of circulating folate, which is a protective factor for adenoma formation, and this may lead to DNA hypomethylation and the formation of abasic sites (20, 32).

Smoking- and alcohol-induced oxidative DNA base modifications and single-strand breaks are repaired by the base excision repair pathway, whereas double-strand breaks are repaired by homologous recombination repair or nonhomologous end-joining. Smoking-induced bulky adducts are repaired by the nucleotide excision repair pathway, and acetaldehyde-induced adducts, such as 1,N^2-propanodeoxyguanosine, are repaired by nucleotide excision repair and homologous recombination repair (31). Interstrand cross-links are also repaired by homologous recombination repair in combination with some nucleotide excision repair proteins (36).

Individuals vary in their ability to repair DNA damage; therefore, genetic variants in genes that code for proteins that participate in the repair of damage induced by smoking and alcohol may modify the effects of these two exposures. This might be particularly relevant for alcohol given the inconsistencies across epidemiologic studies. In this study, we investigated the potential role of single-nucleotide polymorphisms (SNP) in three genes that participate in three pathways that are relevant for the repair of damage induced by smoking and alcohol: *XRCC1* (codons 194 and 399), which participates in base excision repair, *XRCC3* (codon 241), which participates in homologous recombination repair, and *XPD* (codon 751), which participates in nucleotide excision repair and transcription-coupled repair of oxidative damage (37).

Materials and Methods

Study Subjects. All subjects for this study were participants in a University of Southern California/Kaiser study of risk factors for colorectal adenomas and were examined by flexible sigmoidoscopy from 1991 to 1993 (phase I) and 1993 to 1995 (phase II) at either of two southern California Kaiser Permanente Medical Centers (Bellflower and Sunset). Phase I of this study has previously been described (38). Phase II subjects were recruited using the same criteria as phase I. Briefly, eligible English-speaking subjects were 50 to 74 years old, lived in the Los Angeles metropolitan area, had no history of invasive cancer, inflammatory bowel disease, familial polyposis, previous bowel surgery, or symptoms suggestive of gastrointestinal disease. Cases were those subjects with a first-time diagnosis of a histologically confirmed adenoma, whereas controls were subjects with no current or past polyps, individually matched to cases by gender, age (within 5 years), sigmoidoscopy date (within 3 months), and center. All subjects signed a written informed consent approved by the Institutional Review Board, donated a blood sample, and provided demographic information, smoking, family history of cancer, and physical activity among other factors, during an in-person interview. All subjects were interviewed, on average, 5 months after sigmoidoscopy. All participants completed a semiquantitative food frequency questionnaire that inquired about diet in the year before sigmoidoscopy, as previously described (38).

Genotyping Methods. *XRCC1, XRCC3*, and *XPD* genotypes were determined using genomic DNA isolated from peripheral blood lymphocytes and PCR-RFLP assays previously described (39-41).

Statistical Analysis. Frequencies of various demographic variables between cases and controls and between phase I and phase II subjects were compared using contingency tables and χ^2 tests. Within controls, we compared the observed genotype frequencies to those expected under the Hardy-Weinberg law using χ^2 tests. We estimated gene, smoking, and alcohol main effects [odds ratios (OR) and 95% confidence intervals (95% CI)] using unconditional logistic regression adjusting for race and the matching variables age, sex, clinic, and exam date. We had previously found that using unconditional logistic regression adjusting for matching variables led to the same results as a conditional logistic regression analysis, with the added advantage that it allowed us to use all available genotypes in our study set (42). Race-specific ORs were estimated from coefficients obtained by fitting a model that included genotype, race (coded using dummy variables), and the product of gene × race. We investigated smoking by using the following variables: never/ever, never/quit/current, categorical variable for years of smoking (three-level variable, using median among smoking controls as cut point), categorical variable for pack-years smoked (three-level variable, using median among smoking controls as cut point). For alcohol intake, we used the following variables: never/ever drinker and categorical variable of alcohol intake expressed as grams per day (three-level variable, using median among drinking controls as cut point). In estimating the effects of smoking and alcohol, we also considered the following potential confounders: total caloric intake, body mass index, total fruit and vegetable intake, vigorous leisure time activity, and saturated fat intake. Inclusion of these variables did not change the OR estimates by >10%; therefore, we did not keep these variables in the final models. A previous study by our group, based on phase I subjects, also considered fiber intake, nonsteroidal anti-inflammatory drug use, and family history, but none of these variables confounded results for smoking and alcohol (29), and so we did not include these variables in our current models. Data on hormone replacement therapy were only available among phase I subjects at the time of these analyses; among them there was no evidence of confounding by hormone replacement therapy on either smoking or alcohol. Inclusion of smoking status in models to estimate alcohol intake changed estimates by 8% to 9%. Whereas such change does not seem to be large enough to warrant inclusion of this term in the model, we find that it is large enough to change estimates for alcohol and adenoma risk from borderline statistically significant to nonsignificant. Therefore, for reporting of alcohol main effects, we opted to present results from models that adjust for smoking status. We determined the gene mode of action by coding the genotypes assuming either a dominant, recessive, or additive mode of action and then selecting the genetic model that provided the most parsimonious fit to the data using the Aikake information criterion (AIC), which was used to rank models that included each of the different genotype variables (43). For genes with very rare allele frequency, we did not consider the recessive genotype due to instability of model fitting (e.g., *XRCC1* codon 194). We tested for heterogeneity of the ORs by phase (I or II) including interaction variables between genotypes and phase in the logistic regression models. Heterogeneity of the ORs by race or sex was tested in the same manner.

We tested for gene-smoking and gene-alcohol interactions using logistic regression models adjusting for age (continuous), sex, race (White, Latinos, African American, or Asian-Pacific Islander), clinic (Bellflower or Sunset), and exam date. Further adjustment of gene × smoking interaction models for alcohol intake yielded almost identical results; therefore, we did not keep alcohol variables in the models. Alcohol analyses were further adjusted for phase I/II status. We tested for gene-exposure interactions on a multiplicative scale by including in our regression model product terms between the

gene (coded dichotomous assuming either a recessive or dominant mode of action, as we describe in Results) and exposure variables (either dichotomous or three-level variables), in addition to the terms present in the main effect model, and used likelihood ratio tests to compare these models to models that assumed no interaction. For analyses using three-level variables, we used ordered variables and one product term between exposure and genotype. Interaction tests for phase, sex, and all exposure variables were done using likelihood ratio tests with 1 degree of freedom. The interaction test for races was done using likelihood ratio test with 3 degrees of freedom. All analyses were two sided and were done using the statistical software STATA version 8 (STATA Corp., College Station, TX).

Results

Demographic characteristic of phase I and phase II subjects have previously been reported and also summarized in Table 1 (38, 44). Briefly, phase II subjects did not differ from phase I subjects in age, gender, ethnicity, and smoking patterns. However, both cases and controls ascertained during phase I significantly differed from subjects ascertained during phase II about alcohol intake. Phase I subjects tended to drink less alcohol than phase II subjects. Therefore, all analyses of alcohol were further adjusted by phase I/II status.

Smoking, Alcohol, and Colon Adenoma Risk. Estimates for the effects of alcohol and smoking among phase I subjects have previously been reported (29). We now present results combining phase I and phase II subjects. As shown in Table 2, cases and controls showed statistically significant differences in their smoking patterns. Cases were more likely to be ever smokers than controls (OR, 1.4; 95% CI, 1.2-1.8; $P = 0.001$). Compared with subjects who never smoked, risk of colorectal adenomas was positively associated with both smoking duration (years of smoking, $P_{trend} < 0.001$) and smoking intensity (pack-years of smoking, $P_{trend} < 0.001$; Table 1). Ever drinkers were not at a higher risk of colon adenomas (OR, 1.1; 95% CI, 0.9-1.4; $P = 0.328$). Intake of >15 g of ethanol per day (approximately, 15 g = 1 alcoholic drink) was slightly more frequent among cases than controls; however, this difference did not reach statistical significance ($P = 0.198$; Table 2). We found no evidence of heterogeneity of the ORs by sex or race for either smoking or alcohol. In addition, we found no evidence of a synergistic effect of smoking and alcohol (interaction $P = 0.293$).

***XRCC1, XRCC3, XPD*, and Colorectal Adenoma Risk.** Using subjects from phase I (466 cases and 509 controls) and phase II (287 cases and 290 controls) of the study, we genotyped for the *XRCC1* codon 194, *XRCC1* codon 399, *XRCC3* codon 241, and *XPD* codon 751 SNPs, for a total of 753 cases and 799 controls. Allelic and genotype frequencies for the *XRCC1* and *XRCC3* SNPs have previously been reported (44). We now report results for the *XPD* codon 751 SNP. Genotypic frequencies between phase I and phase II controls (all races combined) were not statistically different (Pearson χ^2, $P = 0.292$); however, we observed statistically significant differences among Whites (Pearson χ^2, $P = 0.039$) and Asians (Pearson χ^2, $P = 0.015$). We should note that use of a Bonferroni correction to adjust for the multiple pairwise comparisons made these differences not statistically significant. Genotypic counts are shown in Table 3. We observed deviations from the expected values according to the Hardy-Weinberg law among Asian controls ($P = 0.01$).

For the *XPD* codon 751, we estimated the gene mode of action using model selection with AIC and determined that a recessive mode of action (Lys/Lys and Lys/Gln versus Gln/Gln) was the most parsimonious model. After fitting models with genotype variables that assumed either a dominant, recessive, additive, or no gene model, we ranked models and observed the following order: recessive model (AIC = 2,137.9), additive model (AIC = 2,139.512), no gene model (AIC = 2,140.245), and dominant model (AIC = 2,140.758). Therefore, further analyses were conducted using a dichotomous variable that assumed a recessive mode of action (Table 3). As we previously described, we assumed a dominant mode of action for the *XRCC3* codon 241 SNP, which we now also confirmed with model selection with AIC (44). In addition, as we previously reported, given that both *XRCC1* codon 194 and codon 399 were in linkage disequilibrium ($P < 0.001$), and one of the four possible haplotyes generated by these two SNPs is very rare in our study population (probability of double heterozygotes for codon 194 Trp/codon 399 Gln, <2%), our analysis of the three different haplotypes reduces to an analysis grouping the subjects into relevant genotype combinations, as we have previously reported (44).

Table 1. Demographics and descriptive statistics of cases and controls

Variable	Controls, *n* (%)	Cases, *n* (%)
Mean age at interview (±SD), y	61.2 ± 6.7	61.6 ± 6.8
Race/ethnicity		
White	447 (51)	431 (52)
African American	145 (16)	151 (18)
Latinos	183 (21)	148 (18)
Asian-Pacific Islander	109 (12)	102 (12)
Sex		
Female	307 (34)	312 (37)
Male	594 (66)	543 (64)
Clinic of diagnosis		
Bellflower	568 (63)	535 (63)
Sunset	334 (37)	320 (37)
Study phase		
Phase I	556 (62)	520 (61)
Phase II	346 (38)	335 (39)

Table 2. Smoking, alcohol, and colon adenoma risk

Exposure variable	Controls/cases	OR* (95% CI)	*P*
Smoking status			
Never	362/282	1†	
Ever	483/522	1.4 (1.2-1.8)	0.001
Smoking status			
Never	362/282	1†	
Quit	392/371	1.2 (1.0-1.6)	0.052
Current	91/151	2.2 (1.6-3.0)	<0.001
P_{trend}	<0.001		
Years of smoking			
Never	362/282	1†	
>0-26	244/197	1.1 (0.8-1.4)	0.593
>26	230/315	1.8 (1.4-2.3)	<0.001
P_{trend}	<0.001		
Pack-years smoking			
Never	362/282	1†	
>0-21	243/206	1.1 (0.9-1.4)	0.332
>21	228/304	1.7 (1.3-2.2)	<0.001
P_{trend}	<0.001		
Alcohol intake			
Never	343/301	1†	
Ever	538/525	1.1 (0.9-1.4)	0.328
Alcohol intake (g)			
0	343/301	1†	
Up to 15	401/378	1.1 (0.9-1.4)	0.552
>15	137/147	1.2 (0.9-1.7)	0.190
P_{trend}	0.198		

*Adjusted for age at diagnosis, sex, race (Caucasian, Hispanic, African American, Asian-Pacific Islander), clinic, and exam date. OR estimates of alcohol intake were further adjusted by study phase status (I or II) and smoking status (never, quit, current).
† Reference group.

Table 3. *XPD* codon 751 genotypic frequencies among controls, phase I and phase II subjects combined

	Caucasians, n = 412	African Americans, n = 125	Latinos, n = 163	Asian-Pacific Islander, n = 87
XPD codon 751				
Lys/Lys	176 (43%)	65 (52%)	84 (52%)	66 (76%)
Lys/Gln	183 (44%)	54 (43%)	62 (38%)	16 (18%)
Gln/Gln	52 (13%)	6 (5%)	17 (10%)	5 (6%)
Trp allelic frequency	0.35	0.26	0.29	0.15
HWE* test P	0.681	0.212	0.280	0.01

*Hardy-Weinberg equilibrium test.

As we previously described, whereas no association was observed for the *XRCC3* codon 241 SNP and adenoma risk, an inverse association was found for subjects that carried the *XRCC1* codon 194 Arg/Arg genotype in combination with the *XRCC1* codon 399 Gln/Gln genotype (44). In this study, we tested for heterogeneity of the *XPD* codon 751 ORs by phase I and phase II status and found no evidence that OR estimates differ between these two study groups (likelihood ratio test, P = 0.146); therefore, we present ORs and 95% CIs for both phases combined (Table 4). We tested for heterogeneity of the ORs by age and observed that ORs for the *XPD* codon 751 Gln/Gln genotype differed between subjects younger or older than 61 years (median age among controls; likelihood ratio test, P = 0.04). When considering all races combined, we observed no association between the Gln/Gln genotype and adenoma risk among subjects younger than 61 years (OR, 1.1; 95% CI, 0.6-1.9) but a strong inverse association among subjects older than 61 years (OR, 0.5; 95% CI, 0.3-0.8). A test of heterogeneity of the ORs by race suggested that associations between the *XPD* codon 751 genotype and adenoma risk differ between the different ethnic groups (likelihood ratio test on 3 degrees of freedom, P = 0.009). In particular, we observed a strong inverse association among Latinos, although numbers were small for the Gln/Gln genotype (OR, 0.1; 95% CI, 0.01-0.5; N = 17/1 controls/case with Gln/Gln genotype versus 146/125 controls/case with Lys/Lys or Lys/Gln genotype). Among non-Latino subjects, the OR for the Gln/Gln genotype was 0.9 (95% CI, 0.-1.3), suggesting that the inverse association between the Gln/Gln genotype and adenoma risk is likely driven by the Latino population. We present ORs by ethnicity in Table 4.

***XRCC1, XRCC3, XPD*, and Smoking.** We investigated whether the *XRCC1, XRCC3*, and *XPD* SNPs could modify the effect of smoking. The association between smoking and colorectal adenomas did not differ by *XRCC3* (test of interaction P = 0.465 for never/ever, P = 0.462 for never/quit/current, P = 0.439 for years smoked, and P = 0.311 for pack-years smoked) or *XPD* genotype (test of interaction P = 0.394 for never/ever, P = 0.458 for never/quit/current, P = 0.259 for years smoked, and P = 0.240 for pack-years smoked). However, we found evidence of an *XRCC1* × smoking interaction (Table 5). In particular, we observed that the association between ever smoking and adenoma risk was only observed among subjects who carried the codon 194 Arg/Arg and codon 399 Arg/Arg or Arg/Gln genotypes (test of interaction P = 0.048). For example, the OR for ever versus never smoking among these subjects (''genotype-specific OR'') was 1.6 (95% CI, 1.2-2.0). A similar trend was observed when considering never/quit/current smoking status, pack-years (never, >0-21, >21), and years of smoking (never, >0-26, >26), although analyses using these variables did not reach statistical significance (Table 5).

***XRCC1, XRCC3, XPD*, and Alcohol.** The lack of association between alcohol intake and adenoma risk was not modified by SNPs in the *XRCC1* (test of interaction P = 0.764 for never/ever, P = 0.986 for alcohol g/d) or *XRCC3* (test of interaction P = 0.593 for never/ever, P = 0.954 for alcohol g/d) genes. However, we observed that among subjects who carried the *XPD* codon 751 Gln/Gln genotype, alcohol was associated with adenoma risk (ever drinkers genotype-specific OR, 2.5; 95% CI, 1.2-5.2; test of interaction P = 0.043; Table 6). This *XPD* × alcohol interaction was not further modified by race (three-way interaction P = 0.409) or age (three-way interaction P = 0.562).

Discussion

Our study extends the findings previously reported using subjects from phase I of this sigmoidoscopy-based study about

Table 4. Genotypic frequencies among cases and controls, phase I and phase II subjects

XPD codon 751	Controls (%)	Cases (%)	OR* (95% CI)	P
Lys/Lys	392 (50)	387 (52)	1†	
Lys/Gln	317 (40)	298 (40)	1.0 (0.8-1.2)	0.64
Gln/Gln	80 (10)	55 (7)	0.7 (0.4-1.0)	0.03
Lys/Lys + Lys/Gln	709 (90)	685 (93)	1†	
Gln/Gln	80 (10)	55 (7)	0.7 (0.5-1.0)	0.04
ORs by race (gene × race interaction P = 0.009)‡				
Among Whites				
Lys/Lys + Lys/Gln	359 (87)	351 (89)	1†	
Gln/Gln	52 (13)	44 (11)	0.9 (0.6-1.3)	0.54
Among African American				
Lys/Lys + Lys/Gln	119 (95)	122 (95)	1†	
Gln/Gln	6 (5)	6 (5)	1.0 (0.3-3.1)	0.97
Among Latinos				
Lys/Lys + Lys/Gln	146 (90)	125 (99)	1†	
Gln/Gln	17 (10)	1 (1)	0.1 (0.0-0.5)	0.01
Among Asians				
Lys/Lys + Lys/Gln	82 (94)	80 (98)	1†	
Gln/Gln	5 (6)	2 (2)	0.4 (0.1-1.9)	0.24

*Adjusted for age at diagnosis, sex, race (Caucasian, Hispanic, African American, Asian-Pacific Islander), clinic, and exam date.
† Reference group.
‡ Race-specific ORs were obtained from one single model that included interaction terms between the gene and race dummy variables.

Table 5. *XRCC1* codon 194 and codon 399 and smoking, phase I and phase II subjects combined

Codon 194 Codon 399	Arg/Arg Arg/Arg or Arg/Gln		Arg/Trp or Trp/Trp Arg/Arg or Arg/Gln		Arg/Arg Gln/Gln		Test of interaction *P*
	Controls/Cases	OR* (95% CI)	Controls/Cases	OR* (95% CI)	Controls/Cases	OR* (95% CI)	
Smoking status							
Never	230/173	1†	56/58	1.4 (0.9-2.2)	30/23	1.1 (0.6-1.9)	
Ever	286/333	1.6 (1.2-2.0)	79/79	1.4 (0.9-2.0)	61/34	0.7 (0.5-1.2)	
P_{trend}	0.001		0.973		0.336		0.048
Genotype-specific OR		1.6 (1.2-2.0)		1.0 (0.6-1.6)		0.7 (0.4-1.4)	
Smoking status							
Never	230/173	1†	56/58	1.4 (0.9-2.1)	30/23	1.1 (0.6-1.9)	
Quit	228/237	1.4 (1.0-1.8)	62/59	1.3 (0.9-2.0)	53/26	0.6 (0.4-1.1)	
Current	57/96	2.3 (1.6-3.4)	18/20	1.5 (0.8-2.9)	8/8	1.4 (0.5-3.8)	
P_{trend}	<0.001		0.913		0.852		0.072
Genotype-specific OR	Quit	1.4 (1.0-1.8)		0.9 (0.6-1.6)		0.6 (0.3-1.3)	
	Current	2.3 (1.6-3.4)		1.1 (0.5-2.3)		1.3 (0.4-4.1)	
Years of smoking							
Never	230/173	1†	56/58	1.4 (0.9-2.2)	30/23	1.0 (0.6-1.9)	
>0-26	140/138	1.2 (0.9-1.7)	41/33	1.1 (0.7-1.9)	30/13	0.6 (0.3-1.1)	
>26	141/199	1.9 (1.4-2.6)	36/44	1.7 (1.0-2.8)	30/21	1.0 (0.5-1.7)	
P_{trend}	<0.001		0.560		0.848		0.115
Genotype-specific OR	>0-26	1.2 (0.9-1.7)		0.8 (0.4-1.5)		0.6 (0.2-1.3)	
	>26	1.9 (1.4-2.6)		1.2 (0.7-2.2)		0.9 (0.4-2.0)	
Pack-years of smoking							
Never	230/173	1†	56/58	1.4 (0.9-2.1)	30/23	1.1 (0.6-1.9)	
>0-21	135/127	1.3 (0.9-1.7)	44/38	1.2 (0.7-2.0)	30/10	0.5 (0.2-1.0)	
>21	144/199	1.9 (1.4-2.6)	32/39	1.7 (1.0-2.8)	30/24	1.1 (0.6-2.0)	
P_{trend}	<0.001		0.592		0.888		0.199
Genotype-specific OR	>0-21	1.3 (0.9-1.7)		0.9 (0.5-1.6)		0.4 (0.2-1.1)	
	>21	1.9 (1.4-2.6)		1.2 (0.7-2.2)		1.0 (0.5-2.3)	

*Adjusted for age at diagnosis, sex, race (Caucasian, Hispanic, African American, Asian-Pacific Islander), clinic, and exam date.
† Reference group.

the effects of smoking and alcohol on colorectal adenomas (29). Furthermore, we now provide new evidence that the associations between smoking, alcohol, and adenoma risk seem to be modified by polymorphisms in DNA repair genes. The increased risk associated with smoking seems to be constrained to subjects who carry the *XRCC1* codon 194 Arg/Arg and codon 399 Arg/Arg or Arg/Gln genotypes, although evidence for a gene × smoking interaction only reached statistical significance when considering a dichotomous variable for smoking (ever/never). Whereas only a weak nonsignificant association was observed between alcohol and adenoma risk when ignoring DNA repair genotypes, a stronger significant association was observed among subjects who carry the *XPD* codon 751 Gln/Gln genotype. Again, tests for interaction were only significant when considering a dichotomous category of alcohol intake ($P = 0.04$) and borderline significant ($P = 0.068$) when considering grams of alcohol per day using an ordered categorical three-level variable. Therefore, our results need to be confirmed using larger studies. Furthermore, given that all subjects in our study were examined by flexible sigmoidoscopy, cases were only diagnosed with left-sided adenomas and controls were selected among subjects free of left-sided adenomas. Thus, our findings are only directly applicable to risk of left-sided adenomas.

There are relatively few studies that have investigated the role of DNA repair SNPs and colorectal adenomas (44-48), and of these, only three have explored a potential modifier role for DNA repair SNPs on the effects of alcohol or smoking (46-48). In addition, only three of the previously reported studies investigated the *XPD* codon 751 SNP in relation with adenoma risk. Whereas a sigmoidoscopy-based study reported a nonsignificant inverse association (47), two other studies, one sigmoidoscopy based and the other colonoscopy based, reported a positive association between the Gln allele and adenoma risk (45, 46). These reports are in contrast with our finding of a significant inverse association between the Gln

Table 6. *XPD* codon 751 and alcohol, all subjects combined

Alcohol intake	Gln/Gln		Lys/Lys and Lys/Gln		Test of interaction *P*
	Controls/cases	OR* (95% CI)	Controls/cases	OR* (95% CI)	
Status					
Never	37/15	1†	268/253	2.5 (1.3-4.7)	
Ever	43/40	2.5 (1.2-5.2)	432/422	2.7 (1.4-5.1)	
P_{trend}	0.020		0.547		0.04
Genotype-specific OR		2.5 (1.2-5.2)		1.1 (0.8-1.4)	
Alcohol (g/d)					
0	37/15	1†	268/253	2.5 (1.3-4.7)	
Up to 15	27/24	2.3 (1.0-5.3)	322/311	2.6 (1.4-5.0)	
>15	16/16	2.7 (1.1-6.9)	110/111	2.8 (1.4-5.6)	
P_{trend}	0.028		0.418		0.068
Genotype-specific OR	Up to 15	2.3 (1.0-5.3)		1.1 (0.8-1.3)	
	>15	2.7 (1.1-6.9)		1.2 (0.8-1.6)	

*Adjusted for age at diagnosis, sex, race (Caucasian, Hispanic, African American, Asian-Pacific Islander), clinic, exam date, and study phase status (phase I or II).
† Reference group.

allele and adenoma risk. This discrepancy might indicate that this SNP is in linkage disequilibrium with another marker that might be the causal susceptibility gene and that the linkage disequilibrium pattern might differ between the different study populations. In particular, in our study population, the effects seem to be largely driven by the Latino population.

Regarding the role of *XPD* as a potential effect modifier, whereas Huang et al. (47) found no evidence of an *XPD* × smoking interaction, Bigler et al. (46) reported evidence of an *XPD* × smoking interaction among hyperplastic and adenomatous polyps, when considering the codon 312 and codon 751 polymorphisms combined, but no evidence of an *XPD* × alcohol interaction, which is in contrast with our study where we find some evidence for an *XPD* × alcohol interaction and no evidence of an *XPD* × smoking interaction. A role for *XPD* as a modifier of the effects of alcohol is biologically plausible. An interaction between the XPD codon 751 SNP and alcohol has previously been reported for head and neck cancer (49). The XPD protein is a DNA helicase that is associated with transcription factor IIH and plays a role in basal transcription and nucleotide excision repair (50). The XPD protein enables the DNA helix to open, allowing excision of the DNA fragment containing the damaged base. Alcohol-derived acetaldehyde can induce DNA damage, such as $1,N^2$-propanodeoxyguanosine and intrastrand cross-links, which are repaired by nucleotide excision repair (31, 51). In particular, *in vitro* experiments have shown that nucleotide excision repair–deficient cells (xeroderma pigmentosum cells) are more sensitive to acetaldehyde than nucleotide excision repair–proficient cells, confirming a role for nucleotide excision repair in the repair of acetaldehyde-induced DNA damage (51). Therefore, it is plausible that subtle differences in the activity of the XPD protein might modulate the DNA-damaging effects of alcohol, which include lesions that elicit nucleotide excision repair.

Several reports suggest that the codon 751 Gln allele might be associated with lower repair proficiency of damage induced by UV and chemical carcinogens, although other studies suggest that this allele might confer higher repair proficiency of damage induced by ionizing radiation (52), suggesting a differential effect for this variant on XPD protein function under different exposures.

We found no evidence of a modification of the effects of alcohol or smoking by the *XRCC3* codon 241 SNP, which is in agreement with the report by Tranah et al. (48). To our knowledge, this is the first report of *XRCC1* SNPs as modifiers of the effect of smoking or alcohol on colorectal adenomas; therefore, we cannot compare our findings. Nonetheless, a recent review of the literature found an overall modification of the effect of smoking by *XRCC1* across several cancer types (53). The XRCC1 protein plays a key role in base excision repair where it coordinates all the steps by serving as a scaffold via its interaction with other key base excision repair proteins, such as DNA polymerase β, DNA ligase III, polynucleotide kinase 3′-phosphatase, poly(ADP-ribose) polymerase, APE1, and OGG1 (54). Our results suggest that smoking only has an effect on adenoma risk among carriers of the *XRCC1* codon 194 Arg and codon 399 Arg alleles. Phenotype association studies suggest that the *XRCC1* codon 194 Arg allele may lower the repair proficiency of the XRCC1 protein, whereas codon 399 Arg would be associated with higher repair proficiency (53). Nonetheless, functional studies have failed to find an effect for either SNP (55, 56). Interestingly, a functional effect was reported for the codon 280 SNP (55), which is in linkage disequilibrium with the codon 194 and codon 399 SNPs. Therefore, the results observed in this study might be driven by this SNP, which was not investigated among these subjects. This would explain why the combination of two alleles of apparent opposite effects can increase risk of adenomas in combination with smoking. Further studies should include genotyping of this allele. We found no support for the hypothesis that *XRCC1* might modify the effect of alcohol. This might be due to lack of statistical power to detect an interaction of lower magnitude, our inability to capture all the underlying genetic variations with the two SNPs we selected, or it might be a reflection of a more predominant role for the transcription-coupled repair pathway in repairing alcohol-induced oxidative damage instead of base excision repair. Studies that include more *XRCC1* SNPs and more base excision repair genes will help us better understand this issue.

We found little support for the hypothesis that alcohol might contribute to adenoma formation by acting as a solvent for cigarette smoke carcinogens (32) given that we did not find evidence for a smoking × alcohol interaction. Instead, we found evidence that both smoking and alcohol seem to exert independent effects via their DNA-damaging effects, and that these effects may vary depending on the genetic background of proteins that participate in the base excision repair and nucleotide excision repair pathways. Future studies that include more DNA repair genes in these two pathways, in addition to genes that participate in the metabolism of alcohol, such as alcohol dehydrogenase and aldehyde dehydrogenase, may help further understand the mechanism of action of smoking and alcohol on adenoma formation.

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