

DNA Repair Gene *XRCC1* Polymorphisms, Smoking, and Bladder Cancer Risk

Mariana C. Stern, David M. Umbach, Carla H. van Gils, Ruth M. Lunn, and Jack A. Taylor¹

Molecular and Genetic Epidemiology Section, Laboratory of Molecular Carcinogenesis [M. C. S., C. H. v. G., J. A. T.], Epidemiology Branch [J. A. T.], Biostatistics Branch [D. M. U.], and Laboratory of Computational Biology and Risk Analysis [R. M. L.], National Institute of Environmental Health Sciences, NIH, Research Triangle Park, North Carolina, 27709

Abstract

Bladder cancer is the sixth most common cancer in the United States. The main identified risk factor is cigarette smoking, which is estimated to contribute to up to 50% of new cases in men and 20% in women. Besides containing other carcinogens, cigarette smoke is a rich source of reactive oxygen species (ROS) that can induce a variety of DNA damage, some of which is repaired by the base excision repair (BER) pathway. The *XRCC1* gene protein plays an important role in BER by serving as a scaffold for other repair enzymes and by recognizing single-strand DNA breaks. Three polymorphisms that induce amino acid changes have been found in codon 194 (exon 6), codon 280 (exon 9), and codon 399 (exon 10) of this gene. We tested whether polymorphisms in *XRCC1* were associated with bladder cancer risk and whether this association was modified by cigarette smoking. Therefore, we genotyped for the three polymorphisms in 235 bladder cancer cases and 213 controls who had been frequency matched to cases on age, sex, and ethnicity. We found no evidence of an association between the codon 280 variant and bladder cancer risk [odds ratio (OR), 1.2; 95% confidence interval (CI), 0.6–2.6]. We found some evidence of a protective effect for subjects that carried at least one copy of the codon 194 variant allele relative to those homozygous for the common allele (OR, 0.59; 95% CI, 0.3–1.0). The combined analysis with smoking history suggested a possible gene-exposure interaction; however, the results were not statistically significant. Similarly, for the codon 399 polymorphism, our data suggested a protective effect of the homozygous variant genotype relative to carriers of either one or two copies of the common allele (OR, 0.70; 95% CI, 0.4–1.3), and provided limited evidence, albeit not statistically significant, for a gene-smoking interaction.

Introduction

Bladder cancer is the sixth most common cancer in the United States, with 53,200 incident cases estimated for the year 2000 (1). Smoking constitutes the single most important cause of bladder cancer, with cigarette smokers having 2- to 4-fold higher incidence than nonsmokers (2–6). This risk correlates not only with number of pack-years smoked but also with duration of smoking and the depth of inhalation. Tobacco smoking contributes to 50% of bladder cancer in men and 20% in women (7).

Cigarette smoke is a rich source of chemical carcinogens and ROS.² Chemical carcinogens include polycyclic aromatic hydrocarbons, aromatic amines and *N*-nitroso compounds, which can produce DNA bulky adducts that may lead to DNA damage (8). ROS are present in both the gas-phase and the particulate matter (tar; Ref. 9, 10) and include oxygen radicals, *e.g.*, superoxide radicals (O_2^-) and hydroxyl radicals (OH \cdot), and some derivatives of O_2 that lack unpaired electrons, *e.g.*, hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl; Ref. 11). One report indicated that 5×10^4 radicals are generated with each inhalation from a cigarette (10). Furthermore, through endogenous enzymatic reactions mediated by bacteria and inflammatory cells, *N*-nitroso compounds, such as those in cigarette smoke, can generate nitric oxide radicals that can induce oxidative damage (12). This could explain the finding of infection and inflammation as risk factors for bladder cancer (13). The accumulation of ROS leads to oxidative stress, which is a risk factor for cancer development (14). ROS can initiate lipid peroxidation, oxidize proteins, and cause damage to DNA indirectly or directly (9, 15, 16). Indirect damage includes inactivation of target enzymes, such as those involved in DNA synthesis (17). Direct DNA damage includes DNA strand breaks, creation of abasic sites, and base adduct formation, such as thymine glycol, 5-hydroxymethyluracil and 8-hydroxy-2-deoxyguanosine (15, 17). Up to 4×10^5 oxidatively altered DNA base residues are introduced per day in each cell (17, 18).

Bulky adduct lesions induced by chemical carcinogens are repaired through the nucleotide excision repair (NER) pathway (19). Base damage and DNA single strand breaks are repaired through the BER pathway (20). This pathway is a multistep process that requires the activity of several proteins (17, 20). The *XRCC1* protein is an important component of BER, because it serves as a scaffold for two other proteins, DNA ligase III and POLB and also serves as a single-strand break sensor by its interaction with PARP (21–23). *XRCC1* has been mapped to human chromosome 19q13.2–13.3 (24, 25), and three polymorphisms that lead to amino acid substitutions have been described in codon 194 (exon 6, base 26304 C to T, Arg to Trp),

Received 7/5/00; revised 12/1/00; accepted 12/2/00.

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.

¹ To whom requests for reprints should be addressed, at National Institute of Environmental Health Sciences, P. O. Box 12233, Research Triangle Park, NC 27709. Phone (919) 541-4631; E-mail: taylor@niehs.nih.gov.

² The abbreviations used are: ROS, reactive oxygen species; BER, base excision repair; PARP, poly(ADP-ribose) polymerase; POLB, polymerase β ; OR, odds ratio; CI, confidence interval.

codon 280 (exon 9, base 27466 G to A, Arg to His), and codon 399 (exon 10, base 28152 G to A, Arg to Gln; Ref.26). All three of the polymorphisms occur at residues that are conserved across hamster, mouse, and human (27), but their functional consequences remain unknown.

Assays that measure DNA repair capacity suggest that this function can vary widely among individuals. Cancer patients have lower DNA repair capacities than healthy controls (reviewed in Ref. 28). This finding supports the hypothesis that variants in DNA repair genes could affect cancer susceptibility.

In the present study, we hypothesized that *XRCC1* polymorphisms could affect the capacity of bladder cells to repair ROS-induced DNA damage, and consequently influence bladder cancer risk. Using a case-control study, we tested whether these polymorphisms were associated with bladder cancer risk and examined gene-environment interactions, by testing whether *XRCC1* polymorphisms modified the risk effect of cigarette smoking, a measure of ROS exposure.

Materials and Methods

Patients. Bladder cancer patients ($n = 235$) and control individuals ($n = 213$) were enrolled from the Urology Clinics at Duke University Medical Center and the University of North Carolina Hospitals, as described previously (6, 29). Briefly, cases were urology clinic patients with histologically confirmed transitional cell carcinoma. Controls were urology clinic patients without a history of cancer, frequency matched to cases based on ethnicity, sex, and age (10-year interval). After enrollment, a few controls were excluded given a subsequent diagnosis of prostate cancer. Among controls, the most frequent diagnoses were benign prostate hyperplasia and impotence. All of the individuals were given a questionnaire that detailed their smoking, occupational, and other exposure histories, as well as family history of cancer. After giving written informed consent, subjects provided blood samples collected under protocols approved by the institutional review boards of each participating institution.

Genotype Analysis by PCR-RFLP. DNA was extracted from peripheral blood lymphocytes by standard methods, resuspended in TE buffer (10 mM Tris, 1 mM EDTA), and frozen until use. A multiplex PCR assay was used to amplify fragments surrounding the codon 194 and 399 polymorphisms, as described previously (30). These PCR products were digested with *MspI*, resolved in 3% Metaphor agarose gels (FMC Bioproducts, Rockland, ME), and stained with ethidium bromide. A separate PCR reaction was carried out to amplify the fragment containing the codon 280 polymorphism, as described previously (30). These PCR products were digested with *RspI*, resolved in 2% 3:1 NuSieve agarose gels (FMC Bioproducts), and stained with ethidium bromide.

Statistical Analysis. We used standard methods for $2 \times k$ contingency tables, including Fisher's exact test as appropriate, when analyzing categorical variables without adjustment for covariates (31). These analyses included comparing genotype frequencies between cases and controls as well as examining association of *XRCC1* polymorphisms with tumor grade, tumor stage, or age at diagnosis. We checked for consistency of genotypic frequencies among controls with those expected from the Hardy-Weinberg law using estimates of the disequilibrium coefficient as described by Weir (32). When adjusting for age, sex, or ethnicity and when examining interactions between polymorphisms and smoking, we used standard logistic regression methods (33). We conducted interaction analyses

on the basis of a multiplicative scale. We had too few blacks in our study for reliable inference using their data alone; therefore, we combined blacks and whites in analyses that included ethnicity as a covariate. We examined categorized and continuous versions of pack-years and total number of years-smoked as measures of smoking exposure for gene-environment interaction analyses. As we had previously observed (6), years of smoking was more predictive of bladder cancer risk than other measures of smoking dose; therefore, we present results for that variable.

To examine the combined effects of the continuous smoking variable and the *XRCC1* polymorphisms, we followed an approach that we have used previously (6). This approach fits a series of logistic regression models and compares them to test relevant hypotheses. We used dichotomous genotype variables in these analyses. In the full regression model, log odds of disease was modeled as a separate straight line with respect to years of smoking for each genotype. Under this model, differences between the slopes of the lines represent genotype-exposure interactions, and differences between the intercepts represent the genotype effect among nonsmokers. A two-degree of freedom likelihood ratio test of whether the polymorphism has any effect on risk was made by comparing the full model's likelihood to that of a model in which both genotypes are represented by a single line. A one-degree of freedom test, focused on interaction alone, compares the likelihood of the full model to that of a parallel-lines model in which both lines have a common slope but separate intercepts. All of the tests were two-tailed. All of the analyses were done using the statistical package Egret for Windows (Cytel Software Corporation, Cambridge, MA).

Results

We found no statistically significant differences between cases and controls for the frequency-matched variables sex, ethnicity, and age at interview (Table 1). However, cases did have a slightly higher mean age at interview compared with controls. Cases were more likely to have ever smoked than controls (OR adjusted for age and sex, 4.0; 95% CI, 2.4–6.6). On average, cases smoked 15.9 more pack-years than controls and smoked for 12.7 more years.

***XRCC1* Polymorphisms and Bladder Cancer Risk.** The frequency of the codon 194-*T* allele for cases and controls were 0.06 and 0.09, respectively, for whites and 0.03 and 0.16 for blacks. No subjects carried the codon 194-*TT* genotype. The genotypic frequencies among white controls were consistent with those expected from the Hardy-Weinberg law ($P = 0.36$). The proportion of white subjects homozygous for the codon 194-*C* allele was somewhat higher in cases than in controls (88.3 versus 82.7%; $P = 0.10$; Table 2). This tendency was also observed among blacks (95 versus 77%; $P = 0.13$). The age-, sex-, and ethnicity-adjusted OR for individuals with the variant codon 194-*CT* genotype compared with those with the common *CC* genotype was 0.6 (95% CI, 0.3–1.0; $P = 0.06$) for whites and blacks combined. These results suggest a slight protective effect of the codon 194-*CT* genotype, or conversely a moderate adverse effect of the codon 194-*CC* genotype, on bladder cancer risk.

The codon 280-*A* allele frequency was 0.04 for both cases and controls among whites and 0.05 and 0 for black cases and controls, respectively. No cases carried the codon 280-*AA* genotype. We found that among controls the genotypic frequencies were significantly different from those expected from the Hardy-Weinberg law ($P = 0.002$). However, this effect could be a consequence of the very low allelic frequency, such that

Table 1 Demographic and smoking information on cases and controls

	Cases (n = 235)		Controls (n = 213)		OR (95% CI) ^a	P ^b
	n	%	n	%		
Sex						0.34
Female	53	22.6	40	18.8	ND ^{c,d}	
Male	183	77.4	173	81.2		
Race						0.42
White	215	91.5	198	92.7	ND ^d	
Black	19	8.1	13	6.1		
Other ^e	1	0.4	2	0.9		
Age range (yr)						0.19
<60	60	25.5	67	31.5		
60–70	90	38.3	89	41.8	ND ^d	
>70	85	36.2	57	26.8		
Mean age (SD)	65.6 (10.7)		63.3 (10.4)			0.02 ^f
Smoking history ^g						<0.001
Never	38	16.2	78	36.6	1 ^{ref}	
Quit	137	58.3	109	51.2	3.3 (2.0–5.6)	
Current	60	25.5	26	12.2	6.5 (3.4–12.5)	
Pack-years						<0.001
Nonsmoker	41	17.5	80	37.9	1 ^{ref}	
1–35 pack-years	76	32.5	76	36	2.7 (1.6–4.7)	
>35 pack-years	117	50	55	26.1	6.1 (3.4–10.6)	
Mean pack-years (SD)	40.1 (35.5)		24.2 (32.2)			<0.001 ^f
Years smoked						<0.001
Nonsmoker	37	15.9	77	36.3	1 ^{ref}	
1–34 years smoked	92	39.5	92	43.4	2.9 (1.7–4.9)	
>34 years smoked	104	44.6	43	20.3	6.3 (3.5–11.2)	
Mean years smoked (SD)	29.5 (19.8)		16.8 (16.8)			<0.001 ^f

^a Adjusted for age, sex, and race.

^b χ^2 test for homogeneity of proportions in contingency table.

^c ND, not determined.

^d Cases and controls were frequency-matched on these variables.

^e American Indian/Alaska native (2) and double racial heritage (1).

^f Student's *t* test.

^g Differences in numbers between “Never” and “Pack-years Nonsmoker” and “Years smoked Nonsmoker” are explained by the differences in definition of Nonsmoker within each variable.

the expected number of subjects with the codon 280-AA genotype was 0.3, whereas 2 were observed in our control population. For the codon 280-GA + AA genotype compared with the GG genotype, we found an age-, sex-, and ethnicity-adjusted OR of 1.1 (95% CI, 0.5–2.2; *P* = 0.86) for whites and blacks combined (Table 2). Thus, we saw little evidence of an association between the codon 280 polymorphism and bladder cancer risk.

The codon 399-A allele frequency among white cases was 0.34 and 0.36 among controls. Among blacks, the allele frequencies were 0.26 and 0.15, respectively. We found no significant differences between the observed genotypic frequencies among white controls and those expected from the Hardy-Weinberg law (*P* = 0.92). We found a slight decrease in risk for those individuals who carried the codon 399-AA genotype compared with codon 399-GG genotype, although this difference was not statistically significant (age-, sex-, and ethnicity-adjusted OR, 0.7; 95% CI, 0.4–1.4; *P* = 0.35, for whites and blacks combined; Table 2). Given that the adjusted OR for the codon 399-GA genotype compared with codon 399-GG was 1.1 (95% CI, 0.7–1.6) for whites and blacks combined, we grouped the GG and GA genotypes for further analysis.

Combined Analysis of Codon 194 and 399 Polymorphisms. A combined analysis undertaken to distinguish the relative contributions of the polymorphisms in codons 194 and 399 to bladder cancer risk suggested that the codon 194-CT and the

codon 399-AA genotypes have separate protective effects. Subjects that carried the “protective” codon 194-CT genotype but not the protective codon 399-AA genotype had an OR of 0.6. The same result was observed for subjects that carried the codon 399-AA genotype but not the codon 194-CT genotype (Table 3). We could not examine the joint contribution of these two variants to risk, given the lack of subjects who were both homozygous for the codon 399-A allele and carried the codon 194-T allele.

XRCCI Polymorphisms and Cigarette Smoking Interaction. To assess interaction between the codon 194-CT genotype and smoking, we used as reference the highest risk group: those who had the codon 194-CC genotype and were heavy smokers (Table 4). Although decreasing levels of smoking consistently decreased bladder cancer risk, this effect was more pronounced among codon 194-CT subjects. For example, individuals who smoked 1–34 years and carried the codon 194-CT genotype had an OR of 0.2 (95% CI, 0.1–0.6; whites and blacks combined), whereas those who had the codon 194-CC genotype had an OR of 0.6 (95% CI, 0.3–0.9; whites and blacks combined) relative to the reference group. Similar results were found using categories based on pack-years. These results suggest that the codon 194-T allele may be more protective for bladder cancer in lower-dose smokers than in higher-dose smokers; however, tests for multiplicative interaction were not statistically significant (Table 4). We also looked for the presence of codon

Table 2 Genotypic frequencies and bladder cancer risk

Genotype	Whites				Blacks ^b		Combined	
	Cases (%)	Controls (%)	OR _{adj} ^a	95% CI	Cases	Controls	OR _{adj} ^c	95% CI
Codon 194 ^d								
CC	189 (88)	163 (83)	1 ^{ref}		18 (95)	10 (77)	1 ^{ref}	
CT	24 (12)	34 (17)	0.6	0.4–1.1	1 (5)	3 (23)	0.6	0.3–1.0
TT	0 (0)	0 (0)			0 (0)	0 (0)		
Codon 280 ^e								
GG	198 (92)	180 (92)	1 ^{ref}		17 (89)	13 (100)	1 ^{ref}	
GA	16 (8)	13 (7)	1.1	0.5–2.4	2 (11)	0 (0)	1.2	0.6–2.6
AA	0 (0)	2 (1)			0 (0)	0 (0)		
GG	198 (92)	180 (92)	1 ^{ref}		17 (89)	13 (100)	1 ^{ref}	
GA+AA	16 (8)	15 (8)	1.0	0.5–2.0	2 (11)	0 (0)	1.1	0.5–2.2
Codon 399 ^f								
GG	87 (40)	79 (40)	1 ^{ref}		9 (47)	9 (69)	1 ^{ref}	
GA	106 (50)	92 (47)	1.0	0.6–1.6	10 (53)	4 (31)	1.1	0.7–1.6
AA	21 (10)	26 (13)	0.7	0.4–1.4	0 (0)	0 (0)	0.7	0.4–1.4
GG+GA	193 (90)	171 (87)	1 ^{ref}		19 (100)	13 (100)	1 ^{ref}	
AA	21 (10)	26 (13)	0.7	0.4–1.3	0 (0)	0 (0)	0.7	0.4–1.3

^a Adjusted for age and sex.

^b We omitted the ORs and CIs for black subjects because of the presence of relatively few subjects.

^c Adjusted for age, sex, and ethnicity.

^d Codon 194 (base pair 26304) C to T change, Arginine to Tryptophan amino acid change.

^e Codon 280 (base pair 27466) G to A change, Arginine to Histidine amino acid change.

^f Codon 399 (base pair 28152) G to A change, Arginine to Glutamine amino acid change.

Table 3 XRCCI codon 194 and codon 399 polymorphisms, combined analysis

Codon 194 ^a	Codon 399 ^b	Whites				Blacks		Combined	
		Cases	Controls	OR _{adj} ^c	95% CI	Cases	Controls	OR _{adj} ^d	95% CI
CC	GG+GA	169	137	1 ^{ref}		17	10	1 ^{ref}	
CC	AA	21	26	0.6	0.3–1.2	0	0	0.6	0.3–1.2
CT	GG+GA	25	34	0.6	0.3–1.1	1	3	0.6	0.3–1.0
CT	AA	0	0			0	0		

^a Codon 194 (base pair 26304) C to T change, Arginine to Tryptophan amino acid change.

^b Codon 399 (base pair 28152) G to A change, Arginine to Glutamine amino acid change.

^c Adjusted for age and sex.

^d Adjusted for age, sex, and ethnicity.

194 genotype and smoking interaction using years of smoking as a continuous variable and the logistic regression models described in “Materials and Methods.” A test for any codon 194 association with risk (*i.e.*, combined main effect and interaction) suggested that codon 194 did affect risk ($\chi^2_{2df} = 5.76$; $P = 0.06$). A test of multiplicative interaction alone was not statistically significant ($\chi^2_{1df} = 2.38$; $P = 0.12$). Thus, with these data, we were unable to declare that the observed difference in the codon 194 protective effect between higher- and lower-dose smokers was statistically significant.

As with the variant allele of codon 194, we observed that the reduction in bladder cancer risk associated with decreasing smoking was slightly more pronounced among codon 399-AA subjects. Individuals who smoked 1–34 years and had the codon 399-AA genotype had an OR of 0.3 (95% CI, 0.1–0.8; whites and blacks combined; Table 4), whereas those who had the codon 399-GG+GA genotype had an OR of 0.5 (95% CI, 0.3–0.9; whites and blacks combined). Similar results were found using categories based on pack-years. These results suggest that the codon 399-A allele might be more protective for bladder cancer in lower-dose smokers than in higher-dose smokers; however, the test for

multiplicative interaction was not statistically significant (Table 4). Unlike the result for codon 194, applying years of smoking as a continuous variable provided no significant evidence that codon 399 influenced risk ($\chi^2_{2df} = 3.15$; $P = 0.21$). A test that focused on interaction alone yielded a smaller P but was also nonsignificant ($\chi^2_{1df} = 1.83$; $P = 0.18$).

Our analysis of codon 280 showed no consistent evidence of gene-exposure interaction. However, given the low variant allele frequency we have the least power to examine interaction.

XRCCI Polymorphisms and Prognosis-associated Variables. We examined whether cases with and without each of the variant genotypes differed for age at diagnosis, using the median age of diagnosis (63 years) as the cutoff point. We did not find statistically significant differences among cases with and without the codon 194-CT genotype ($P = 0.61$), the codon 399-GG+GA genotypes ($P = 0.91$) or the codon 280-GA+AA genotypes ($P = 0.54$). We also found no statistically significant differences for the variables grade and stage among cases with and without the codon 194-CT genotype ($P = 0.63$ for grade and 0.63 for stage), the codon 399-GG+GA genotypes ($P =$

Table 4 XRCC1 codon 194 and 399 polymorphisms and smoking, stratified analysis

Years smoked	Genotype	Whites				Test for Interaction P_{adj}^a	Blacks		Combined		
		Cases	Controls	OR _{adj} ^a	95% CI		Cases	Controls	OR _{adj} ^b	95% CI	Test for interaction P_{adj}^b
Codon 194 ^c											
>34	CC	83	36	1 ^{ref}			7	3	1 ^{ref}		
>34	CT	12	4	1.3	0.4–4.2		0	0	1.3	0.4–4.3	
1–34	CC	72	65	0.5	0.3–0.9		9	5	0.6	0.3–0.9	
1–34	CT	11	20	0.3	0.1–0.6	0.21	0	1	0.2	0.1–0.6	0.16
0	CC	33	61	0.2	0.1–0.3		1	2	0.2	0.1–0.3	
0	CT	2	10	0.1	0.0–0.4	0.25	1	2	0.1	0.0–0.3	0.26
Codon 399 ^d											
>34	GG+GA	84	37	1 ^{ref}			7	3	1 ^{ref}		
>34	AA	11	3	1.6	0.4–6.3		0	0	1.7	0.4–6.4	
1–34	GG+GA	77	73	0.5	0.3–0.8		9	6	0.5	0.3–0.9	
1–34	AA	6	12	0.2	0.1–0.7	0.18	0	0	0.3	0.1–0.8	0.16
0	GG+GA	32	60	0.2	0.1–0.4		2	4	0.2	0.1–0.4	
0	AA	3	11	0.1	0.0–0.3	0.18	0	0	0.1	0.0–0.3	0.17

^a Adjusted for age and sex.

^b Adjusted for age, sex, and ethnicity.

^c Codon 194 (base pair 26304) C to T change, Arginine to Tryptophan amino acid change.

^d Codon 399 (base pair 28152) G to A change, Arginine to Glutamine amino acid change.

0.97 for grade and 0.43 for stage) or the codon 280-GA+AA genotypes ($P = 0.97$ for grade and 0.56 for stage).

Discussion

The XRCC1 protein has no known catalytic activity but serves to orchestrate BER via its role as a central scaffolding protein for DNA ligase III (22, 34), DNA POLB (35), and PARP (23), and via its function in recognizing and binding to single-strand breaks (36, 37). XRCC1 appears to be an essential protein, with the knockout of XRCC1 being embryonic lethal in the mouse (38). XRCC1 complements the deficiencies of the Chinese hamster ovary (CHO) mutant EM9 cells whose defects include sensitivity to ionizing radiation and alkylating agents (39, 40), increased double-strand breaks and sister chromatid exchange (41, 42), and decreased recombinational and single-strand break repair.

The XRCC1 codon 194 and codon 280 polymorphisms are located in the linker region that separates the DNA POLB interacting domain from the PARP-interacting domain (27, 35). The codon 399 polymorphism is located on the COOH-terminal side of the PARP-interacting domain, within the BRCT1 domain (23). BRCT domains are homologous to the COOH-terminal region of the breast cancer susceptibility gene BRCA1 and are thought to mediate specific protein-protein interactions. BRCT domains are present in a number of DNA-damage response proteins, including p53 (43). Although all of the three polymorphisms lead to amino acid substitutions, there is no direct data on their functional consequences.

We found some support for the hypothesis that the XRCC1 polymorphisms affect bladder cancer risk. The codon 194 variant allele provided a modest protective effect, of borderline statistical significance, and the more frequently observed codon 399 variant also appeared to provide protection, although this finding was not statistically significant. We did not observe any individuals who carried the protective genotype at both loci. Interestingly, the variant alleles at codons 194 and 399 each had a protective effect in the absence of the other, suggesting that they may independently affect risk. The codon 280 polymorphism showed relatively little association with risk, although

the low frequency of this variant allele limited our power to detect a difference.

When we considered smoking status as a surrogate for increased oxidative damage, the protective effects of the codon 194 and codon 399 genotypes could be consistently demonstrated in both the moderate smoking strata and the nonsmoking strata but not in the strata comprised of heavy smokers. This finding held whether we used pack-years, years-smoked, or other smoking classification schemes, although such classifications are highly correlated. Formal tests for gene-smoking interaction were suggestive, although not statistically significant. Such an interaction is biologically plausible; although the protective effect of XRCC1 variant alleles in repairing oxidative damage might be evident in people with low levels of endogenous and exogenous oxidative damage, subtle differences in repair efficiencies between the common and variant alleles might be overwhelmed by the high levels of oxidative damage present in heavy smokers. Similar patterns of genotype effects at low smoking exposure but not at high smoking exposure have been observed for GSTM1 and lung cancer risk (44) and CYP1A1 (45), in which subtle genotypic differences in detoxification are made irrelevant at high dose.

We found that these polymorphisms did not affect age of onset of bladder tumors, or the aggressiveness of the disease, given the lack of association with grade and stage of the tumors. Other bladder cancer risk factors could modify the effect of these polymorphisms, such as family history of cancer (46–48) or the presence of other genetic susceptibility markers, such as variants for metabolism enzymes that we have previously characterized for this study (6, 29). The activation and detoxification of carcinogens and ROS, as well as the repair of the damage induced by these compounds, are important aspects in carcinogenesis. Both are under genetic control, and there is increasing evidence of genetic variation of these pathways in the human population. Therefore, integrating information on allelic variants of these genes may be useful in determining groups of individuals at highest risk from environmental exposure. In the case of XRCC1, the analyses of possible interactions with GSTM1 polymorphisms are appropriate given the role of

this enzyme in detoxifying products of oxidative stress (49). We are currently conducting analyses to investigate family history and multiple gene effects on exposure-related cancer risk.

A study by Sturgis *et al.* (50) of 203 cases of head and neck cancer and 424 controls reported increased risk for the codon 194-CC genotype (OR, 1.3; 95% CI, 0.8–2.2). Recalculation of this result with the codon 194-CC group as the reference gives an OR of 0.8 (95% CI, 0.5–1.3) for codon 194-CT+TT subjects, comparable with our result for bladder cancer. However, their data showed evidence of increased risk associated with the codon 399 homozygous variant (AA) genotype compared with those with one or more copies of the common allele (OR, 1.6; 95% CI, 1.0–2.6). In a study by Lunn *et al.* (30), Taiwanese women who had the codon 399-GA or AA genotype were more likely to have detectable levels of aflatoxin B1-DNA adducts than women with the GG genotype (OR, 2.9; 95% CI, 1.3–6.4). Women who carried the codon 194-CT or TT genotypes were less likely to have detectable levels of adducts, although this association was not statistically significant (OR, 0.6; 95% CI, 0.3–1.3). In this same study, an association was found for carriers of the codon 399-AA genotype and increased glyophorin A mutations, an association that was stronger among smokers. In a study by Duell *et al.* (51), healthy subjects who carried the codon 399-AA genotype showed slightly higher mean sister chromatid exchange (SCE) frequencies. This increase was more pronounced among current smokers (OR, 1.4; 95% CI, 1.2–1.7) compared with never smokers who carried the codon 399-GG genotype. Also, in this same study, an association was found between the presence of polyphenol DNA adducts and one or more copies of the codon 399-A variant allele. This association was more evident among subjects older than 65 years, which suggested a possible interaction with age.

Given the lack of direct functional measures of DNA repair capacity associated with the different polymorphisms, it is difficult to integrate the sometimes divergent results of the existing studies on XRCC1. One of the limitations of our study is the use of hospital-based controls, which could introduce a possible selection bias if the clinical conditions of these subjects were associated with genotype. However, we find that possibility unlikely in our study. The most common diagnoses among controls were impotence and incontinence, and there is no evidence to suggest that DNA repair genes might be involved in the etiology of these conditions. In addition, the allelic frequencies observed among our white control group are remarkably similar to those observed in both the hospital-based study by Sturgis *et al.* (50) and to population-based studies by Lunn *et al.* (30) and our own group,³ which suggests that there was no apparent selection bias in the control group.

If the observation that XRCC1 variant alleles in codon 194 and 399 decrease bladder cancer risk is true, we can suggest three alternative hypotheses to interpret these findings: first, that these gene variants independently confer improved function to the XRCC1 protein. Although one might expect that better functioning alleles would be the more common, the fact that their frequency in the population is lower than the “less protective” alleles could be a reflection of recent appearance during evolution or the absence of a selective advantage to reproductive fitness. A second hypothesis could be that these variants diminish the efficiency of the XRCC1 protein but still

provide decreased risk from cancer. Under this scenario, cells with excessive oxidative damage that carry such variants would have decreased ability to repair DNA damage and might be more likely to undergo apoptosis or senescence. Such decreased efficiency could be an “advantage” if it avoided the transmission and clonal expansion of mutations that could arise during BER, which is an error-prone repair process. A third hypothesis is that another polymorphic gene might be in linkage disequilibrium with XRCC1. Interestingly, the ERCC2 (XPD) gene also maps to chromosome 19q13.2 (25, 52) adjacent to XRCC1, which maps to 19q13.2–13.3 (24, 25). ERCC2 is involved in nucleotide excision repair of bulky adducts, such as those induced by many of the carcinogens in cigarette smoke. We are currently examining possible associations between ERCC2 polymorphisms and bladder cancer risk.

Acknowledgments

We thank Lyle Lansdell and Patty Blanton for data handling, sample retrieval, and management; Drs. James Mohler, David Paulson, and Cary Robertson for patient enrollment; and Drs. Glinda Cooper and Jane Schroeder for critical review of the manuscript.

References

- Greenlee, R. T., Murray, T., Bolden, S., and Wingo, P. A. Cancer statistics, 2000. *CA Cancer J. Clin.*, 50: 7–33, 2000.
- Hartge, P., Silverman, D., Hoover, R., Schairer, C., Altman, R., Austin, D., Cantor, K., Child, M., Key, C., Marrett, L. D., *et al.* Changing cigarette habits and bladder cancer risk: a case-control study. *J. Natl. Cancer Inst.*, 78: 1119–1125, 1987.
- Burch, J. D., Rohan, T. E., Howe, G. R., Risch, H. A., Hill, G. B., Steele, R., and Miller, A. B. Risk of bladder cancer by source and type of tobacco exposure: a case-control study. *Int. J. Cancer*, 44: 622–628, 1989.
- Clavel, J., Cordier, S., Boccon-Gibod, L., and Hemon, D. Tobacco and bladder cancer in males: increased risk for inhalers and smokers of black tobacco. *Int. J. Cancer*, 44: 605–610, 1989.
- Morrison, A. S., Buring, J. E., Verhoek, W. G., Aoki, K., Leck, I., Ohno, Y., and Obata, K. An international study of smoking and bladder cancer. *J. Urol.*, 131: 650–654, 1984.
- Taylor, J. A., Umbach, D. M., Stephens, E., Castranio, T., Paulson, D., Robertson, C., Mohler, J. L., and Bell, D. A. The role of N-acetylation polymorphisms in smoking-associated bladder cancer: evidence of a gene-gene-exposure three-way interaction. *Cancer Res.*, 58: 3603–3610, 1998.
- IARC, Tobacco smoking. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Lyon, France: IARC, 1986.
- Vineis, P., Talaska, G., Malaveille, C., Bartsch, H., Martone, T., Sithisarankul, P., and Strickland, P. DNA adducts in urothelial cells: relationship with biomarkers of exposure to arylamines and polycyclic aromatic hydrocarbons from tobacco smoke. *Int. J. Cancer*, 65: 314–316, 1996.
- Pryor, W. A. Cigarette smoke radicals and the role of free radicals in chemical carcinogenicity. *Environ Health Perspect.*, 105 (Suppl. 4): 875–882, 1997.
- Kiyosawa, H., Suko, M., Okudaira, H., Murata, K., Miyamoto, T., Chung, M. H., Kasai, H., and Nishimura, S. Cigarette smoking induces formation of 8-hydroxydeoxyguanosine, one of the oxidative DNA damages in human peripheral leukocytes. *Free Radic. Res. Commun.*, 11: 23–27, 1990.
- Halliwell, B., and Cross, C. E. Oxygen-derived species: their relation to human disease and environmental stress. *Environ Health Perspect.*, 102 (Suppl. 10): 5–12, 1994.
- Bartsch, H., Ohshima, H., Pignatelli, B., and Calmels, S. Endogenously formed N-nitroso compounds and nitrosating agents in human cancer etiology. *Pharmacogenetics*, 2: 272–277, 1992.
- Shirai, T., Fradet, Y., Huland, H., Bollack, C., Droller, M., Janknegt, R., Jones, P., and Kagawa, S. The etiology of bladder cancer—are there any new clues or predictors of behavior? *Int. J. Urol.*, 2 (Suppl. 2): 64–75, 1995.
- Bankson, D. D., Kestin, M., and Rifai, N. Role of free radicals in cancer and atherosclerosis. *Clin. Lab. Med.*, 13: 463–480, 1993.
- Joenje, H. Genetic toxicology of oxygen. *Mutat. Res.*, 219: 193–208, 1989.
- Floyd, R. A. The role of 8-hydroxyguanine in carcinogenesis. *Carcinogenesis (Lond.)*, 11: 1447–1450, 1990.
- Friedberg, E. C., Walker, G. C., and Siede, W. DNA Repair and Mutagenesis. Washington, DC: ASM Press, 1995.

³ C. H. van Gils, R. M. Bostick, M. C. Stern, and J. A. Taylor. Polymorphisms in DNA repair gene XRCC1, diet, and prostate cancer risk, manuscript in preparation.

18. Roldan-Arjona, T., Wei, Y. F., Carter, K. C., Klungland, A., Anselmino, C., Wang, R. P., Augustus, M., and Lindahl, T. Molecular cloning and functional expression of a human cDNA encoding the antimutator enzyme 8-hydroxyguanine-DNA glycosylase. *Proc. Natl. Acad. Sci. USA*, *94*: 8016–8020, 1997.
19. Benhamou, S., and Sarasin, A. Variability in nucleotide excision repair and cancer risk: a review. *Mutat. Res.*, *462*: 149–158, 2000.
20. Wilson, D. M., III, and Thompson, L. H. Life without DNA repair. *Proc. Natl. Acad. Sci. USA*, *94*: 12754–12757, 1997.
21. Caldecott, K. W., McKeown, C. K., Tucker, J. D., Ljungquist, S., and Thompson, L. H. An interaction between the mammalian DNA repair protein XRCC1 and DNA ligase III. *Mol. Cell. Biol.*, *14*: 68–76, 1994.
22. Caldecott, K. W., Aoufouchi, S., Johnson, P., and Shall, S. XRCC1 polypeptide interacts with DNA polymerase β and possibly poly(ADP-ribose) polymerase, and DNA ligase III is a novel molecular 'nick-sensor' *in vitro*. *Nucleic Acids Res.*, *24*: 4387–4394, 1996.
23. Masson, M., Niedergang, C., Schreiber, V., Muller, S., Menissier-de Murcia, J., and de Murcia, G. XRCC1 is specifically associated with poly(ADP-ribose) polymerase and negatively regulates its activity following DNA damage. *Mol. Cell. Biol.*, *18*: 3563–3571, 1998.
24. Thompson, L. H., Bachinski, L. L., Stallings, R. L., Dolf, G., Weber, C. A., Westerveld, A., and Siciliano, M. J. Complementation of repair gene mutations on the hemizygous chromosome 9 in CHO: a third repair gene on human chromosome 19. *Genomics*, *5*: 670–679, 1989.
25. Mohrenweiser, H. W., Carrano, A. V., Fertitta, A., Perry, B., Thompson, L. H., Tucker, J. D., and Weber, C. A. Refined mapping of the three DNA repair genes, *ERCC1*, *ERCC2*, and *XRCC1*, on human chromosome 19. *Cytogenet. Cell Genet.*, *52*: 11–14, 1989.
26. Shen, M. R., Jones, I. M., and Mohrenweiser, H. Nonconservative amino acid substitution variants exist at polymorphic frequency in DNA repair genes in healthy humans. *Cancer Res.*, *58*: 604–608, 1998.
27. Lamerdin, J. E., Montgomery, M. A., Stilwagen, S. A., Scheidecker, L. K., Tebbs, R. S., Brookman, K. W., Thompson, L. H., and Carrano, A. V. Genomic sequence comparison of the human and mouse *XRCC1* DNA repair gene regions. *Genomics*, *25*: 547–554, 1995.
28. Mohrenweiser, H. W., and Jones, I. M. Variation in DNA repair is a factor in cancer susceptibility: a paradigm for the promises and perils of individual and population risk estimation? *Mutat. Res.*, *400*: 15–24, 1998.
29. Bell, D. A., Taylor, J. A., Paulson, D. F., Robertson, C. N., Mohler, J. L., and Lucier, G. W. Genetic risk and carcinogen exposure: a common inherited defect of the carcinogen-metabolism gene glutathione S-transferase M1 (*GSTM1*) that increases susceptibility to bladder cancer. *J. Natl. Cancer Inst.*, *85*: 1159–1164, 1993.
30. Lunn, R. M., Langlois, R. G., Hsieh, L. L., Thompson, C. L., and Bell, D. A. XRCC1 polymorphisms: effects on aflatoxin B1-DNA adducts and glycoprotein A variant frequency. *Cancer Res.*, *59*: 2557–2561, 1999.
31. Fleiss, J. L. *Statistical methods for rates and proportions*. New York: John Wiley & Sons, 1981.
32. Weir, B. S. *Genetic Data Analysis II*. Sunderland, Massachusetts: Sinauer Associates, Inc. Publishers, 1996.
33. Hosmer, D. W., and Lemeshow, S. *Applied logistic regression*. New York: John Wiley & Sons, 1989.
34. Nash, R. A., Caldecott, K. W., Barnes, D. E., and Lindahl, T. XRCC1 protein interacts with one of two distinct forms of DNA ligase III. *Biochemistry*, *36*: 5207–5211, 1997.
35. Kubota, Y., Nash, R. A., Klungland, A., Schar, P., Barnes, D. E., and Lindahl, T. Reconstitution of DNA base excision-repair with purified human proteins: interaction between DNA polymerase β and the XRCC1 protein. *EMBO J.*, *15*: 6662–6670, 1996.
36. Rice, P. A. Holding damaged DNA together. *Nat. Struct. Biol.*, *6*: 805–806, 1999.
37. Marintchev, A., Mullen, M. A., Maciejewski, M. W., Pan, B., Gryk, M. R., and Mullen, G. P. Solution structure of the single-strand break repair protein XRCC1 N-terminal domain. *Nat. Struct. Biol.*, *6*: 884–893, 1999.
38. Tebbs, R. S., Flannery, M. L., Meneses, J. J., Hartmann, A., Tucker, J. D., Thompson, L. H., Cleaver, J. E., and Pedersen, R. A. Requirement for the *Xrcc1* DNA base excision repair gene during early mouse development. *Dev. Biol.*, *208*: 513–529, 1999.
39. Caldecott, K., and Jeggo, P. Cross-sensitivity of γ -ray-sensitive hamster mutants to cross-linking agents. *Mutat. Res.*, *255*: 111–121, 1991.
40. Thompson, L. H., Brookman, K. W., Jones, N. J., Allen, S. A., and Carrano, A. V. Molecular cloning of the human *XRCC1* gene, which corrects defective DNA strand break repair and sister chromatid exchange. *Mol. Cell. Biol.*, *10*: 6160–6171, 1990.
41. Carrano, A. V., Minkler, J. L., Dillehay, L. E., and Thompson, L. H. Incorporated bromodeoxyuridine enhances the sister-chromatid exchange and chromosomal aberration frequencies in an EMS-sensitive Chinese hamster cell line. *Mutat. Res.*, *162*: 233–239, 1986.
42. Hoy, C. A., Fuscoe, J. C., and Thompson, L. H. Recombination and ligation of transfected DNA in CHO mutant EM9, which has high levels of sister chromatid exchange. *Mol. Cell. Biol.*, *7*: 2007–2011, 1987.
43. Callebaut, I., and Mornon, J. P. From BRCA1 to RAP1: a widespread BRCT module closely associated with DNA repair. *FEBS Lett.*, *400*: 25–30, 1997.
44. London, S. J., Daly, A. K., Cooper, J., Navidi, W. C., Carpenter, C. L., and Idle, J. R. Polymorphism of glutathione S-transferase M1 and lung cancer risk among African-Americans and Caucasians in Los Angeles County, California. *J. Natl. Cancer Inst.*, *87*: 1246–1253, 1995.
45. Nakachi, K., Imai, K., Hayashi, S., and Kawajiri, K. Polymorphisms of the *CYP1A1* and glutathione S-transferase genes associated with susceptibility to lung cancer in relation to cigarette dose in a Japanese population. *Cancer Res.*, *53*: 2994–2999, 1993.
46. Kantor, A. F., Hartge, P., Hoover, R. N., and Fraumeni, J. F., Jr. Familial and environmental interactions in bladder cancer risk. *Int. J. Cancer*, *35*: 703–706, 1985.
47. Kramer, A. A., Graham, S., Burnett, W. S., and Nasca, P. Familial aggregation of bladder cancer stratified by smoking status. *Epidemiology*, *2*: 145–148, 1991.
48. Sturgeon, S. R., Hartge, P., Silverman, D. T., Kantor, A. F., Linehan, W. M., Lynch, C., and Hoover, R. N. Associations between bladder cancer risk factors and tumor stage and grade at diagnosis. *Epidemiology*, *5*: 218–225, 1994.
49. Strange, R. C., and Fryer, A. A. Chapter 19. The glutathione S-transferases: influence of polymorphism on cancer susceptibility. *IARC Sci. Publ.*, *148*: 231–249, 1999.
50. Sturgis, E. M., Castillo, E. J., Li, L., Zheng, R., Eicher, S. A., Clayman, G. L., Strom, S. S., Spitz, M. R., and Wei, Q. Polymorphisms of DNA repair gene *XRCC1* in squamous cell carcinoma of the head and neck. *Carcinogenesis (Lond.)*, *20*: 2125–2129, 1999.
51. Duell, E. J., Wiencke, J. K., Cheng, T. J., Varkonyi, A., Zuo, Z. F., Ashok, T. D., Mark, E. J., Wain, J. C., Christiani, D. C., and Kelsey, K. T. Polymorphisms in the DNA repair genes *XRCC1* and *ERCC2* and biomarkers of DNA damage in human blood mononuclear cells. *Carcinogenesis (Lond.)*, *21*: 965–971, 2000.
52. Smeets, H., Bachinski, L., Coerwinkel, M., Schepens, J., Hoeijmakers, J., van Duin, M., Grzeschik, K. H., Weber, C. A., de Jong, P., Siciliano, M. J., and Wieringa, B. A long-range restriction map of the human chromosome 19q13 region: close physical linkage between CKMM and the *ERCC1* and *ERCC2* genes. *Am. J. Hum. Genet.*, *46*: 492–501, 1990.