Short Communication

DNA Repair Gene *XRCC3* Codon 241 Polymorphism, Its Interaction with Smoking and *XRCC1* Polymorphisms, and Bladder Cancer Risk

Mariana C. Stern,[1] David M. Umbach, Ruth M. Lunn,[2] and Jack A. Taylor[3]

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

Abstract

DNA repair efficiency varies among individuals, with reduced repair capacity as a risk factor for various cancers. This variability could be partly explained by allelic variants for different DNA repair genes. We examined the role of a common polymorphism in the *XRCC3* gene (codon 241: threonine to methionine change) and bladder cancer risk. This gene plays a role in the homologous recombination pathway, which repairs double-strand breaks. The functional consequences of the *XRCC3* codon 241 polymorphism are still unknown. We hypothesized that this polymorphism could affect repair of smoking-associated DNA damage and could thereby affect bladder cancer risk. We genotyped 233 bladder cancer cases and 209 controls who had been frequency matched to cases on age, sex, and ethnicity. We observed little evidence of a positive association between subjects who carried at least one copy of the codon 241 Met allele and bladder cancer (odds ratio: 1.3; 95% confidence interval: 0.9–1.9). Among heavy smokers, individuals with the Met allele had about twice the risk of those without it; however, a test of interaction was not statistically significant ($P = 0.26$). Previously, we observed in these subjects an association between bladder cancer risk and allelic variants of the *XRCC1* gene, which is involved in the repair of base damage and single-strand breaks. In this study, we found some evidence for a gene-gene interaction between the *XRCC1* codon 194 and *XRCC3* codon 241 polymorphisms ($P = 0.09$) and some support for a possible gene-gene-smoking three-way interaction ($P = 0.08$).

Received 3/30/01; revised 6/3/02; accepted 6/5/02.

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] Present address: Department of Preventive Medicine, University of Southern California, Keck School of Medicine, Norris Comprehensive Cancer Center, Los Angeles, CA 90089.

[2] Present address: Toxicology Operations Branch, National Institute of Environmental Health Sciences, P. O. Box 12233, Research Triangle Park, NC 27709.

[3] To whom correspondence should be addressed, at National Institute of Environmental Health Sciences, P. O. Box 12233, Research Triangle Park, NC 27709. Phone (919) 451-4631; E-mail: taylor@niehs.nih.gov.

Introduction

DNA repair plays a key role in carcinogenesis through the removal of DNA damage induced by endogenous and environmental sources. We are interested in polymorphic genes involved in the repair of smoking-induced DNA damage and in their association with bladder cancer risk. The main risk factor for bladder cancer is cigarette smoking, which can increase risk up to four times (1, 2).

Cigarette smoke is a rich source of chemical carcinogens and ROS[4]. Chemical carcinogens form bulky adducts on DNA, whereas ROS can induce base damage, SSBs, and DSBs (3). Bulky adducts are repaired through the nucleotide excision repair pathway (4). ROS-induced base damage and SSBs are repaired through the base excision repair pathway (5), whereas DSBs can be repaired by either HRR or nonhomologous end joining (6). We have previously reported an association between bladder cancer risk and allelic variations in the XRCC1 gene, which is involved in BER (7).

In this study, we focused on the *XRCC3* gene, which is required for efficient repair of DSBs through the HRR pathway (8), for repair of DNA cross-linking (9), and for chromosomal segregation (10). During HRR, the XRCC3 protein interacts with the Rad51 protein, enabling Rad51 protein multimers to assemble at the site of damage (9, 11). Furthermore, Rad51 has also been found to colocalize with the XRCC1 protein after base damage, suggesting coordination between XRCC1-dependent SSB repair and recombination events during DNA replication (12).

A common polymorphism in the *XRCC3* gene in codon 241 results in a Thr to Met substitution (13). Using a bladder cancer case-control study, we analyzed a possible association between the *XRCC3* codon 241 polymorphism and bladder cancer and a possible interaction with cigarette smoking as a measure of ROS exposure. We also tested for an *XRCC1-XRCC3* interaction and a possible effect modification of this interaction on the effect of cigarette smoking.

Materials and Methods

Subjects. 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 (2, 7). Briefly, cases had histologically confirmed transitional cell carcinoma. Controls were urology patients without a history of cancer from the same clinics, the most frequent diagnoses being benign prostate hypertrophy, impotence, and urinary incontinence. Controls were frequency matched to cases based on ethnicity

[4] The abbreviations used are: ROS, reactive oxygen species; SSB, single-strand break; DSB, double-strand break; HRR, homologous recombination repair; OR, odds ratio; CI, confidence interval.

Table 1 Genotypic frequencies and *XRCC3* codon 241 polymorphism and smoking combined analysis[a]

Smoking	*XRCC3* codon 241	Whites and blacks				
		Cases $n = 233$	Controls $n = 209$	OR_{adj}[b]	95% CI	Test for interaction *P*
Nonsmokers and smokers combined	Thr/Thr	90 (39%)	94 (45%)	1[d]		
	Thr/Met	110 (47%)	91 (44%)	1.2	0.8–1.9	NA[c]
	Met/Met	33 (14%)	24 (11%)	1.5	0.8–2.7	NA
Pack-years						
0	Thr/Thr	19	39	1[ref]		
0	Thr/Met + Met/Met	23	39	1.2	0.5–2.5	
1–35	Thr/Thr	35	28	3.3	1.5–7.3	
1–35	Thr/Met + Met/Met	40	48	2.4	1.1–4.9	0.34
>35	Thr/Thr	36	27	3.9	1.8–8.6	
>35	Thr/Met + Met/Met	79	27	8.3	3.9–18	0.26

[a] Two individuals, one case and one control, both carriers of the Met allele, were missing pack-years information.
[b] Adjusted for age and sex and ethnicity.
[c] NA, not applicable.
[d] ref, reference group.

Table 2 *XRCC1* and *XRCC3* polymorphisms and bladder cancer risk, combined analysis[a]

XRCC1	*XRCC3* codon 241	Whites and blacks				
		Cases	Controls	OR_{adj}[b]	95% CI	Test of interaction *P*
Codon 194[c]						
Arg/Arg	Thr/Met + Met/Met	123	93	1[e]		
Arg/Arg	Thr/Thr	83	76	0.8	0.6–1.3	
Arg/Trp[d]	Thr/Met + Met/Met	20	19	0.8	0.4–1.6	
Arg/Trp[d]	Thr/Thr[d]	6	18	0.2	0.1–0.6	0.09
Codon 399						
Arg/Arg + Arg/Gln	Thr/Met + Met/Met	128	97	1[ref]		
Arg/Arg + Arg/Gln	Thr/Thr[d]	83	83	0.8	0.5–1.1	
Gln/Gln[d]	Thr/Met + Met/Met	15	15	0.8	0.4–1.6	
Gln/Gln[d]	Thr/Thr[d]	6	11	0.4	0.2–1.2	0.61

[a] One white case, carrying the Thr/Thr genotype, and three white controls, carrying the Thr/Met or Met/Met genotype, had to be excluded from the analysis because of lack of *XRCC1* genotype information.
[b] Adjusted for age, sex, and ethnicity.
[c] No subjects had Trp/Trp genotype.
[d] Protective genotypes.
[e] ref, reference group.

(black, white), sex, and age at interview (10-year interval). After giving written informed consent, all individuals were administered a questionnaire that detailed their smoking exposure history and 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. The DNA segment surrounding codon 241 was amplified by PCR using 50 ng of DNA in a final volume of 15 μl containing 1× PCR buffer, 0.2 mM dNTPs, 2 mM $MgCl_2$, 0.8 μM forward primer (at 17,724 bp: 5′-TTGGGGCCTCTTTGAGA-3′), 0.8 μM reverse primer (at 18,258 bp: 5′-AACGGCTGAGGGTCTTCT-3′), and 0.5 units of AmpliTaq Gold (Perkin-Elmer, Foster City, CA). PCR reactions were carried out in a Perkin-Elmer 9700 thermocycler with an initial denaturation step of 8 min at 94°C, followed by 30 cycles at 94°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 1 min. PCR products were digested with *Nla*III restriction enzyme (New England Biolabs, Beverly, MA), resolved in 3% NuSieve 3:1 agarose gels (FMC Bioproducts, Rockland, ME), and stained with ethidium bromide. *Nla*III recognizes an invariant restriction site around bp 17,963, which serves as an internal control for complete enzyme digestion. The *XRCC3* codon 241 Met-allele creates a *Nla*III restriction site at bp 18,067, a site that is not present in the codon 241 Thr-allele.

Statistical Analysis. We used standard methods for 2 × k contingency tables, including Fisher's exact test, as appropriate, to analyze categorical variables without adjustment for covariates. We checked among controls for differences between the observed genotypic frequencies and those expected under the Hardy-Weinberg law using estimates of the disequilibrium coefficient (14). When adjusting for age, sex, or ethnicity, and when examining interactions between the polymorphism and smoking, we used standard logistic regression methods (15). We tested for interaction on a multiplicative scale. Given the small number of blacks in our study, data from blacks are included with whites in combined OR estimates after including ethnicity as a covariate. For gene-environment interaction analyses, we used categorized versions of pack-years as measures of smoking exposure and years of smoking as a continuous variable as described previously (2). We examined the combined effects of the continuous smoking variable and the *XRCC3* genotype and the combined effects of smoking with the *XRCC3* and *XRCC1* genotypes using an approach that we have used previously (2, 7). This approach fits a series of logistic regression models and compares them, testing relevant hypotheses with likelihood ratio tests. Within a model, differences

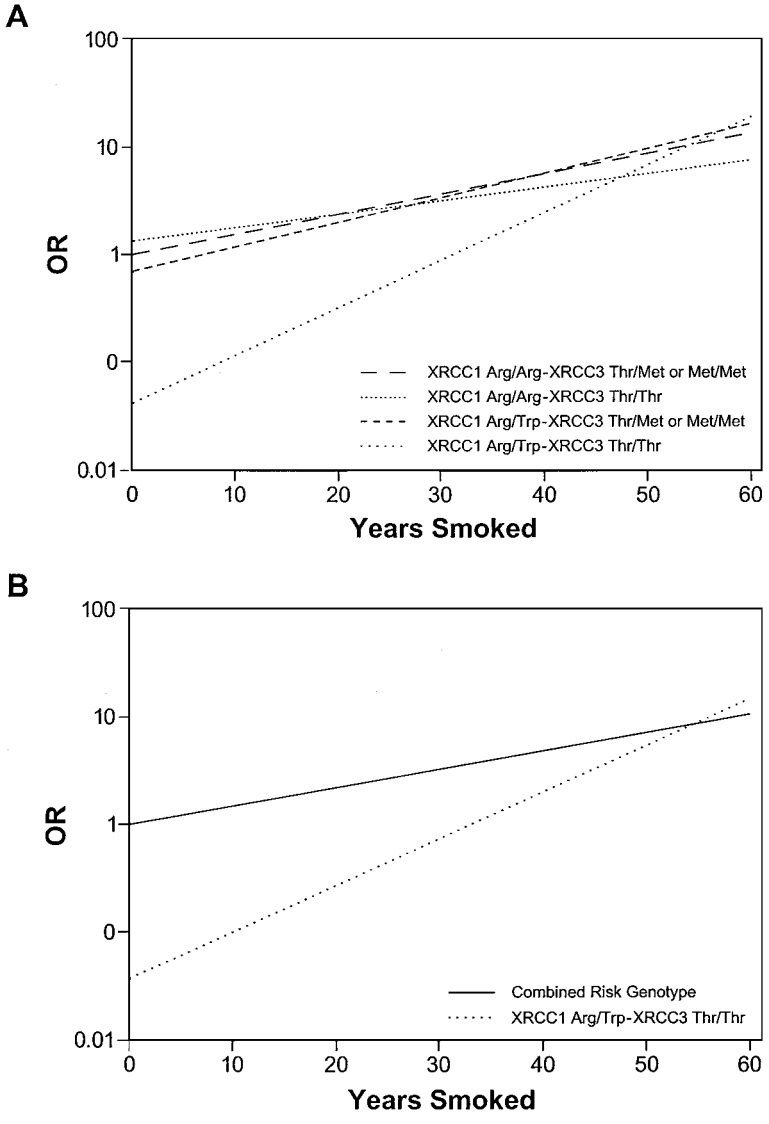

Fig. 1. Estimated ORs for four possible combinations of *XRCC3* codon 241 and *XRCC1* codon 194 genotypes plotted against years of smoking from a model in which slope and intercept are fitted separately for each line (*A*) or from a simplified model where the genotypic combination of *XRCC3* codon 241 Thr/Met or Met/Met and *XRCC1* codon 194 Arg/Trp was fitted as one line (*B*; *dotted line*), and all other genotypic combinations were plotted as a single line referred as combined risk genotype (*solid line*).

between slopes represent genotype-exposure interactions, and differences between intercepts represent genotype effects among nonsmokers. All tests were two-tailed. All analyses were done using the statistical package Egret for Windows (Cytel Software Corporation, Cambridge, MA).

Results

Demographic and smoking information on this study have been previously reported (2, 7). Estimates and tests reported below are for whites and blacks combined, adjusted for age, sex, and ethnicity.

Codon 241 Polymorphism and Bladder Cancer Risk. The frequency of the codon 241 Met allele among cases and controls were 0.39 and 0.34, respectively, for whites and 0.26 and 0.12 for blacks. Among white or black controls, there were no significant differences between the observed genotypic frequencies and those expected under the Hardy-Weinberg law ($P = 0.78$ for whites and $P = 0.61$ for blacks). The OR for bladder cancer for those individuals with one copy of the codon 241 Met allele compared with those with none was 1.2 (95% CI: 0.8–1.9), and the corresponding OR for those with two copies of the Met allele was 1.5 (95% CI: 0.8–2.7; Table 1). For the remaining analysis, we combined subjects who carried one or two copies of the Met allele (OR = 1.3; 95% CI = 0.9–1.9).

Genotype and Smoking. Among never smokers, we observed little association between subjects with the codon 241 Thr/Met or Met/Met genotypes and bladder cancer (Table 1). Among moderate smokers (1–35 pack-years), subjects with the codon 241 Thr/Met or Met/Met genotypes had a slightly lower OR than those homozygous for the Thr allele, with considerable overlap in the 95% CI for the two estimates (Table 1), giving a gene effect within this smoking strata of 0.7 (95% CI: 0.4–1.4). However, among the high-level smoking category (>35 pack-years), the codon 241 Thr/Met or Met/Met genotypes were associated with a higher bladder cancer risk than that of subjects that smoked the same amount but who carried the Thr/Thr genotype (Table 1), giving a gene effect within this smoking strata of 2.1 (adjusted 95% CI: 1.1–4.2). Tests of interaction that compared the gene effect in each of the smoking categories to that among never smokers were not statistically

significant (Table 1). A test for *XRCC3*-smoking interaction using years of smoking as a continuous variable detected no interaction ($\chi^2_{1df} = 1.1$; $P = 0.29$).

XRCC1 and *XRCC3*. We examined whether the *XRCC3* polymorphism had differential effects depending on *XRCC1* genotype using data we had previously obtained on *XRCC1* polymorphisms in these subjects (7). Our earlier data on *XRCC1* supported an inverse association between the codon 194 Arg/Trp genotype and bladder cancer (no subjects carried the Trp/Trp genotype) and a separate inverse association between the codon 399 Gln/Gln genotype and cancer risk. For this analysis, we used as a reference the genotype group with the largest sample size, which was the highest risk group, *i.e.*, subjects with the *XRCC1* codon 194 Arg/Arg and the *XRCC3* codon 241 Thr/Met or Met/Met genotypes (Table 2). Our results suggest that the inverse association between the codon 241 Thr/Thr genotype and cancer risk might be stronger among subjects who also carry the *XRCC1* codon 194 Arg/Trp genotype than among those with the *XRCC1* codon 194 Arg/Arg genotype, however, the test for interaction did not reach statistical significance ($P = 0.09$). The combined presence of the *XRCC1* codon 399 and *XRCC3* codon 241 polymorphisms showed a similar pattern, but the test for interaction was far from statistical significance ($P = 0.61$).

XRCC1, XRCC3, and Smoking. To examine if the combined presence of the *XRCC1* codon 194 and *XRCC3* polymorphisms could modify the effect of cigarette smoking on bladder cancer risk, we used years of smoking as a predictor of bladder cancer risk in the logistic regression model, and we fitted separate regression lines for each of the four combined *XRCC1-XRCC3* genotypes (fitted lines in Fig. 1*A*). This four-line model fit our data significantly better than a model with a single regression line for all four genotype combinations ($\chi^2_{6df} = 12.9$; $P = 0.04$). The fitted regression lines suggested that individuals who carry the *XRCC1* codon 194 Trp/Arg genotype and the *XRCC3* codon 241 Thr/Thr genotype (protective genotype) have a different smoking dose response from any of the other three combined genotypes (risk genotypes). Therefore, we examined a data-suggested hypothesis. The three risk genotypes did not appear to differ in their smoking dose response ($\chi^2_{4df} = 2.1$; $P = 0.72$), thus, we fitted a common smoking dose-response line for the combined at-risk genotypes (Fig. 1*B*). This combined smoking dose response appeared quite distinct from that of the single protective genotype ($\chi^2_{2df} = 10.8$; $P = 0.004$). In particular, the slopes of the two lines appeared to differ, suggesting a possible gene-gene-smoking three way interaction ($\chi^2_{1df} = 3.0$; $P = 0.08$).

Discussion

Two case-control studies have found a significant positive association between the *XRCC3* codon 241 Met allele and melanoma (16) and bladder cancer (17), whereas a lung cancer study found no evidence of an association among Caucasians and a nonsignificant positive association among African-Americans (18).

Our analysis of the *XRCC3* gene alone, ignoring smoking and the *XRCC1* genotype, provides little evidence of a positive association between the *XRCC3* codon 241 Met variant allele and bladder cancer. Among heavy smokers, subjects with the Met allele had roughly twice the risk of those who had comparable smoking histories but who did not carry the Met allele; however, the test for interaction with smoking exposure did not reach statistical significance. In contrast, Matullo *et al.* (17) reported that the positive association between the Met allele and cancer risk seemed stronger among ex-smokers and nonsmokers than among current smokers. However, like our study, they found little evidence for gene-smoking interaction.

The *XRCC1* and *XRCC3* combined analyses suggested that the observed small difference in risk between different *XRCC3* genotypes may be more relevant among individuals with the *XRCC1* codon 194 Arg/Trp genotypes, however, the test for interaction did not reach statistical significance ($P = 0.09$). The combined *XRCC1* codon 194 Arg/Trp and *XRCC3* codon 241 Thr/Thr genotypes had a stronger inverse association with bladder cancer among lower-dose smokers than higher-dose smokers, although again the test for interaction did not reach statistical significance ($P = 0.08$). Genotype risks converged at high-smoking dose, which differs from what was observed with *XRCC3* alone, where genotype risks differed most among heavier smokers. We should note that the size of our study has very limited power for assessing three-way interactions; therefore, our findings must be interpreted with caution and need to be validated in larger studies. As yet, no studies have directly examined the function of the *XRCC3* polymorphism. However, a statistically significant association was described between the codon 241 Met variant and higher frequency of kinetochore-positive micronuclei among healthy cigarette smokers.[5] Given that cigarette smoking induces micronucleus formation (19), the reported association suggests that this allele may have impaired function. An *XRCC1-XRCC3*-smoking interaction seems plausible. Both proteins play important roles in the repair of strand breaks. The XRCC1 protein can detect and repair SSBs (20), which may convert to DSBs after DNA replication (21). The XRCC3 protein plays a role in the repair of DSBs through the HRR by its interaction with the Rad51 protein (8, 9, 11). Furthermore, XRCC1 has been found to colocalize with Rad51 after DNA damage (12). Evidence for such an interaction between a base excision repair pathway and a recombination repair gene has been found in studies in the yeast *Schizosaccharomyces pombe* (22). Our findings highlight the importance of integrating the information from multiple genes in relevant pathways to better identify people at risk from environmental exposures.

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; Sue Edelstein for graphic design; and Drs. Carla H. van Gils and Jane C. Schroeder for critical review of this manuscript.

References

1. 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.

2. 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.

3. Pryor, W. A., Hales, B. J., Premovic, P. I., and Church, D. F. The radicals in cigarette tar: their nature and suggested physiological implications. Science (Wash. DC), *220:* 425–427, 1983.

4. Benhamou, S., and Sarasin, A. Variability in nucleotide excision repair and cancer risk: a review. Mutat. Res., *462:* 149–158, 2000.

5. Wilson, D. M., III, and Thompson, L. H. Life without DNA repair. Proc. Natl. Acad. Sci. USA, *94:* 12754–12757, 1997.

[5] R. M. Lunn, D. S. Rupa, L. Hasegawa, I. M. Jones, C. L. Thompson, D. A. Eastmond, and D. A. Bell. DNA repair gene polymorphisms, HPRT mutations, and micronuclei, submitted for publication.

6. Chu, G. Double strand break repair. J. Biol. Chem., *272:* 24097–24100, 1997.

7. Stern, M. C., Umbach, D. M., van Gils, C. H., Lunn, R. M., and Taylor, J. A. DNA repair gene XRCC1 polymorphisms, smoking, and bladder cancer risk. Cancer Epidemiol. Biomark. Prev., *10:* 125–131, 2001.

8. Pierce, A. J., Johnson, R. D., Thompson, L. H., and Jasin, M. XRCC3 promotes homology-directed repair of DNA damage in mammalian cells. Genes Dev., *13:* 2633–2638, 1999.

9. Liu, N., Lamerdin, J. E., Tebbs, R. S., Schild, D., Tucker, J. D., Shen, M. R., Brookman, K. W., Siciliano, M. J., Walter, C. A., Fan, W., Narayana, L. S., Zhou, Z. Q., Adamson, A. W., Sorensen, K. J., Chen, D. J., Jones, N. J., and Thompson, L. H. XRCC2 and XRCC3, new human Rad51-family members, promote chromosome stability and protect against DNA cross-links and other damages. Mol. Cell, *1:* 783–793, 1998.

10. Griffin, C. S., Simpson, P. J., Wilson, C. R., and Thacker, J. Mammalian recombination-repair genes XRCC2 and XRCC3 promote correct chromosome segregation. Nat. Cell Biol., *2:* 757–761, 2000.

11. Bishop, D. K., Ear, U., Bhattacharyya, A., Calderone, C., Beckett, M., Weichselbaum, R. R., and Shinohara, A. Xrcc3 is required for assembly of rad51 complexes *in vivo*. J. Biol. Chem., *273:* 21482–21488, 1998.

12. Taylor, R. M., Moore, D. J., Whitehouse, J., Johnson, P., and Caldecott, K. W. A cell cycle-specific requirement for the XRCC1 BRCT II domain during mammalian DNA strand break repair. Mol. Cell. Biol., *20:* 735–740, 2000.

13. 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.

14. Weir, B. S. Genetic Data Analysis II. Sunderland, Massachusetts: Sinauer Associates, Inc. Publishers, 1996.

15. Hosmer, D. W., and Lemeshow, S. Applied logistic regression. New York: John Wiley & Sons, 1989.

16. Winsey, S. L., Haldar, N. A., Marsh, H. P., Bunce, M., Marshall, S. E., Harris, A. L., Wojnarowska, F., and Welsh, K. I. A variant within the DNA repair gene XRCC3 is associated with the development of melanoma skin cancer. Cancer Res., *60:* 5612–5616, 2000.

17. Matullo, G., Guarrera, S., Carturan, S., Peluso, M., Malaveille, C., Davico, L., Piazza, A., and Vineis, P. DNA repair gene polymorphisms, bulky DNA adducts in white blood cells and bladder cancer in a case-control study. Int. J. Cancer, *92:* 562–567, 2001.

18. David-Beabes, G. L., Lunn, R. M., and London, S. J. No association between the XPD (Lys751G1n) polymorphism or the XRCC3 (Thr241Met) polymorphism and lung cancer risk. Cancer Epidemiol. Biomark. Prev., *10:* 911–912, 2001.

19. Tomanin, R., Ballarin, C., Nardini, B., Mastrangelo, G., and Sarto, F. Influence of smoking habit on the frequency of micronuclei in human lymphocytes by the cytokinesis block method. Mutagenesis, *6:* 123–126, 1991.

20. 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.

21. Shinohara, A., and Ogawa, T. Homologous recombination and the roles of double-strand breaks. Trends Biochem. Sci., *20:* 387–391, 1995.

22. Memisoglu, A., and Samson, L. Contribution of base excision repair, nucleotide excision repair, and DNA recombination to alkylation resistance of the fission yeast *Schizosaccharomyces pombe*. J. Bacteriol., *182:* 2104–2112, 2000.

DNA Repair Gene *XRCC3* Codon 241 Polymorphism, Its Interaction with Smoking and *XRCC1* Polymorphisms, and Bladder Cancer Risk

Mariana C. Stern, David M. Umbach, Ruth M. Lunn, et al.

Cancer Epidemiol Biomarkers Prev 2002;11:939-943.