Null Results in Brief

No Association between the *XPD* (Lys751G1n) Polymorphism or the *XRCC3* (Thr241Met) Polymorphism and Lung Cancer Risk[1]

Gloria L. David-Beabes,[2] Ruth M. Lunn, and Stephanie J. London

Epidemiology Branch [S. J. L.], Laboratory of Pulmonary Pathobiology [G. L. D-B., S. J. L.], and Toxicology Operations Branch [R. M. L.], National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709

Introduction

DNA repair helps to protect the genome from tobacco-induced damage. Reduced DNA repair capacity may predispose individuals to lung cancer (1). Polymorphisms in genes encoding DNA repair proteins may modulate susceptibility to tobacco lung carcinogens.

Bulky DNA adducts induced by chemical carcinogens in cigarette smoke are repaired through the nucleotide excision repair pathway that includes the *XPD* gene. An *XPD* variant in exon 23 leading to a Lys751G1n amino acid substitution has been associated with reduced DNA repair capacity (2). DNA strand breaks generated by reactive oxygen species in tobacco smoke may be repaired through the homology-directed double-stranded DNA break repair pathway that includes XRCC3. A nucleotide substitution in exon 7 of the *XRCC3* gene results in an amino acid change Thr241Met. There are few data on these polymorphisms in relation to lung cancer (3, 4).

To investigate the possible association between the *XPD* Lys751G1n and *XRCC3* Thr241Met polymorphisms and the risk of lung cancer among Caucasians and African Americans, we analyzed DNA samples from a case-control study of 331 incident lung cancer cases and 687 population controls in Los Angeles County, California.

Materials and Methods

Detailed descriptions of the methods of subject enrollment and study population have been published previously (5). The *XPD* codon 751 genotypes were determined using a PCR-RFLP technique as described previously (2). The *XRCC3* codon 241 genotypes were also determined using PCR-RFLP. The *XRCC3* polymorphic site was amplified from 50 ng of DNA using 0.8 μM of each primer (forward primer, 5′-TTGGGGCCTCTTTGAGA-3′; reverse primer, 5′-AACGGCTGAGGGTCTTCT-3′), 200 μM of each deoxynucleotide triphosphate, 0.5 unit of Taq polymerase (Promega, Madison, WI) and Taqstart antibody (Sigma Chemical Co., St. Louis, MO), and 2 mM $MgCl_2$ in 1× PCR buffer (Promega). The PCR cycling conditions consisted of initial denaturation at 94°C for 4 min followed by 30 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 1 min, and a final step at 72°C for 7 min. The products were digested with *Nla*III (New England Biolabs, Beverly, MA) and resolved on 3% metaphor agarose gels (BioWhittaker Molecular Applications, Rockland, ME). The three possible genotypes are defined by distinct banding patterns: (*a*) Thr/Thr (239 and 313 bp); (*b*) Thr/Met (105, 208, 239, and 313 bp); and (*c*) Met/Met (105, 208, and 239 bp). As a quality control, both assays were repeated on 5% of the samples, and the replicates were 100% concordant.

ORs[3] and 95% CIs were calculated by unconditional logistic regression using SAS version 8 (SAS Institute, Cary, NC). All ORs are adjusted for age and sex and for smoking (the natural logarithm of pack-years and a product term for pack-years and years since quitting smoking). Results in all subjects were also adjusted for ethnicity.

Results and Discussion

The frequency of the *XPD* Gln-751 allele was 0.347 for Caucasians and 0.250 for African Americans. The *XRCC3* Met-241 allele frequency was 0.382 for Caucasians and 0.231 for African Americans. The frequencies were consistent with previous studies (2–4) and were in Hardy-Weinberg equilibrium among controls in both ethnic groups.

Our data do not support any appreciable association between *XPD* codon 751 or *XRCC3* codon 241 genotypes and lung cancer risk in either ethnic group (Table 1). We combined the data for Caucasians and African Americans because we observed no evidence of heterogeneity by ethnic group ($P > 0.30$ for both *XPD* and *XRCC3*), and found no association. In addition, we observed no evidence of effect modification by cell type (adenocarcinoma, squamous plus small cell carcinoma, and all other types). We found no effect modification by amount smoked, dichotomized at the median value for all smokers (data not shown).

The limitations of this study are those of other case-control studies—primarily selection bias and population stratification. Among all subjects, we had 80% power (two-sided test of significance, $\alpha = 0.05$) to detect an OR as low as 1.5 for carriers of one Gln allele or 1.8 for carriers of two Gln alleles relative to none for *XPD*. For *XRCC3*, we had 80% power to detect an OR as low as 1.5 for carriers of one Met allele and 1.7 for carriers of two Met alleles relative to none. Thus the study had good power for these modest associations with these common polymorphisms. Our data in African Americans and Caucasians are in agreement with two recent studies showing no substantive evidence of a differential risk for lung cancer ac-

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[2] To whom requests for reprints should be addressed, at P. O. Box 12233, MD C2-04, Research Triangle Park, NC 27709. Phone: (919) 541-4876; Fax: (919) 541-4133; E-mail: davidbe1@niehs.nih.gov.

[3] The abbreviations used are: OR, odds ratio; CI, confidence interval.

Table 1 Lung cancer risk in relation to *XPD* codon 751 and *XRCC3* codon 241 polymorphisms

Genotype	Caucasians			African Americans			Combined		
	Cases	Controls	OR (95% CI)*	Cases	Controls	OR (95% CI)	Cases	Controls	OR (95% CI)
XPD codon 751									
Lys/Lys	67	197	1.00	79	130	1.00	146	327	1.00
Lys/Gln	77	198	0.97 (0.62–1.52)	63	91	1.08 (0.66–1.76)	140	289	1.03 (0.74–1.43)
Gln/Gln	34	58	1.34 (0.74–2.42)	11	13	1.03 (0.40–2.65)	45	71	1.31 (0.80–2.15)
Lys/Gln + Gln/Gln	111	256	1.06 (0.70–1.61)	74	104	1.07 (0.67–1.71)	185	360	1.08 (0.80–1.47)
XRCC3 codon 241									
Thr/Thr	76	175	1.00	90	136	1.00	166	311	1.00
Thr/Met	78	210	0.93 (0.60–1.43)	54	88	0.90 (0.55–1.48)	132	298	0.92 (0.67–1.28)
Met/Met	24	68	0.94 (0.51–1.77)	9	10	1.67 (0.57–4.87)	33	78	1.08 (0.64–1.83)
Thr/Met + Met/Met	102	278	0.93 (0.62–1.41)	63	98	0.98 (0.61–1.56)	165	376	0.95 (0.70–1.30)
Total	178	453		153	234		331	687	

* ORs and 95% CIs are adjusted for age, sex, and smoking (the natural logarithm of pack-years and the product of the natural logarithm of pack-years and years since quitting smoking). The combined ORs are also adjusted for ethnicity.

cording to *XPD* codon 751 (3, 4) or *XRCC3* codon 241 (4) polymorphisms.

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References

1. Wei, Q., Cheng, L., Hong, W. K., and Spitz, M. R. Reduced DNA repair capacity in lung cancer patients. Cancer Res., *56:* 4103–4107, 1996.

2. Lunn, R. M., Helzlsouer, K. J., Parshad, R., Umbach, D. M., Harris, E. L., Sanford, K. K., and Bell, D. A. XPD polymorphisms: effects on DNA repair proficiency. Carcinogenesis (Lond.), *21:* 551–555, 2000.

3. Spitz, M. R., Wu, X., Wang, Y., Wang, L. E., Shete, S., Amos, C. I., Guo, Z., Lei, L., Mohrenweiser, H., and Wei, Q. Modulation of nucleotide excision repair capacity by XPD polymorphisms in lung cancer patients. Cancer Res., *61:* 1354–1357, 2001.

4. Butkiewicz, D., Rusin, M., Enewold, L., Shields, P. G., Chorazy, M., and Harris, C. C. Genetic polymorphisms in DNA repair genes and risk of lung cancer. Carcinogenesis (Lond.), *22:* 593–597, 2001.

5. 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. (Bethesda), *87:* 1246–1253, 1995.

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