Null Results in Brief

No Association Between *SOD2* or *NQO1* Genotypes and Risk of Bladder Cancer

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Introduction

SOD2 encodes manganese superoxide dismutase, a mitochondrial enzyme that protects the cell against damage from superoxide free radicals. A common polymorphism, a T → C transition (Val16Ala), was shown to alter the manganese superoxide dismutase mitochondrial targeting sequence (1). Animal studies have linked reduction in manganese superoxide dismutase activity to increased DNA damage and higher incidence of cancer (2). *NQO1* encodes NAD(P)H dehydrogenase (quinone), a flavoprotein that also protects the cell against cytotoxicity, by promoting the two-electron reduction of quinoid compounds to hydroquinones (3). A common polymorphism, a C → T transition (Pro187Ser), produces significant reductions in enzyme activity in human colon cancer cells (3). Therefore, in the case-control study reported here, we examined the associations between polymorphisms in *SOD2* and *NQO1*, cigarette smoking, and bladder cancer risk.

Materials and Methods

Bladder cancer patients (n = 239) and control individuals (n = 215) were enrolled from the Urology Clinics at Duke University Medical Center and the University of North Carolina Hospitals as previously described (4). 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 intervals). The most common diagnoses among controls were benign prostatic hypertrophy and impotence. Case patients and control subjects were interviewed by trained nurse-interviewers using a structured questionnaire that detailed their smoking, occupational, and other exposure histories.

DNA was extracted from peripheral blood lymphocytes by standard methods and frozen until used. Genotype at each of the two polymorphic sites was determined using allele-specific primer extension reactions followed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Individuals homozygous for the minor allele, heterozygous, or homozygous for the common allele could always be clearly distinguished, with peaks correctly corresponding to predicted masses. Eight subjects were missing genotype data for either *SOD2* or *NQO1*: three cases and one control, all smokers, were missing *SOD2*; two cases and one control, both smokers, plus one case nonsmoker, were missing *NQO1*. Thus, 236 cases and 214 control subjects were available for analysis of *SOD2* and *NQO1*, respectively. Information on smoking status was not available for one individual, a case. Unconditional logistic regression models were used to estimate odds ratios (OR) and 95% confidence intervals (95% CI). Multivariable models included the variables listed in Table 1 legend. Tests for interaction were based on likelihood ratio tests that compared models with and without product terms representing the variables of interest. All statistical tests were two sided.

Results

Participants were mostly males (77% of cases and 81% of controls) and White (91% of cases and 93% of controls). The mean age was 65.7 years (SD = 10.7 years) for cases and 63.3 years (SD = 10.3 years) for controls. We detected no deviations from Hardy-Weinberg equilibrium among cases or controls (P > 0.51 for each locus). The frequency of the variant allele in the entire sample was 50.2% for *SOD2* and 17.6% for *NQO1*.

SOD2 genotype was not associated with bladder cancer risk (Table 1), although the data showed evidence of a small increase in risk among individuals with Ala/Ala or Val/Ala genotypes compared with those with Val/Val. Among smokers, individuals with the Val/Ala genotype showed some indication of increased risk (OR, 1.7; 95% CI, 1.0-2.9), but an elevated OR was not observed for those with the Ala/Ala genotype, thus showing no trend with increasing number of putative risk alleles. *NQO1* genotype was not associated with bladder cancer risk (Table 1), although risk estimates for the variant genotype Ser/Ser were unstable due to small numbers. When the association was examined by strata of smoking, a statistically insignificant positive elevated OR was observed among nonsmokers with the presumptive high-risk allele. The latter finding was based on relatively small numbers of cases and controls. For *NQO1*, the statistical power of a two-tailed level 5% Fisher's Exact test using the entire sample (239 cases, 215 controls) to detect an OR of 2.0 for carriers of the variant allele compared with those homozygous for the common allele is 94%; using smokers alone (196 cases, 135 controls), power is 83%. For *SOD2*, the corresponding power values are 80% for the entire sample and 65% for smokers alone (minimal OR detectable with 80% power using smokers alone is ~2.3). The results of our study were not altered appreciably by exclusion of the 95 females or 37 non-Whites from the analyses.

Received 7/30/04; revised 9/27/04; accepted 10/13/04.

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Table 1. Multivariate-adjusted ORs for *NQO1* and *SOD2* polymorphism genotypes and bladder cancer risk

	Total population		Never smokers		Smokers	
	Cases/controls	OR (95% CI)	Cases/controls	OR (95% CI)	Cases/controls	OR (95% CI)
SOD2						
Val/Val	54/57	1.0 (reference)	10/18	1.0 (reference)	44/39	1.0 (reference)
Val/Ala	122/103	1.4 (0.8-2.2)	18/42	0.9 (0.3-2.3)	104/61	1.7 (1.0-2.9)
Ala/Ala	59/54	1.4 (0.8-2.6)	12/19	1.3 (0.2-3.9)	47/35	1.2 (0.7-2.3)
Val/Ala or Ala/Ala	181/157	1.4 (0.9-2.3)	30/61	1.0 (0.4-2.5)	151/96	1.5 (0.9-2.5)
NQO1						
Pro/Pro	156/150	1.0 (reference)	23/59	1.0 (reference)	133/91	1.0 (reference)
Pro/Ser	70/58	1.1 (0.7-1.7)	13/19	1.8 (0.7-4.5)	57/39	0.9 (0.5-1.6)
Ser/Ser	9/6	1.4 (0.5-4.4)	3/1	8.0 (0.7-91.2)	6/5	0.7 (0.2-2.5)
Pro/Ser or Ser/Ser	79/64	1.1 (0.7-1.7)	16/20	2.1 (0.9-5.1)	63/44	0.9 (0.5-1.5)

NOTE: Multivariate models included age (continuous variable), sex (female, male), race (White, other), and smoking (duration in 10-year categories, when possible).

Few studies have examined polymorphisms in these metabolism genes with respect to bladder cancer risk (5-9), and they have shown inconsistent findings. Animal experiments and *in vitro* studies suggest that proteins encoded by *SOD2* and *NQO1* can modulate DNA damage from reactive oxygen species and, hence, cancer risk (1, 2). We found no association with *SOD2* or *NQO1* genotype and bladder cancer risk, although the possibility of small increases in risk among individuals with the putative risk alleles cannot be excluded because of the small number of participants in some of the genotype categories.

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Cancer Epidemiol Biomarkers Prev 2005;14:753-754.