A Population-Based Case-Control Study of the *XRCC1 Arg399Gln* Polymorphism and Susceptibility to Bladder Cancer

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Abstract

Cigarette smoking is the major cause of bladder cancer. Constituents in tobacco smoke can induce oxidative DNA damage requiring base excision repair. The *Arg399Gln* polymorphism in the DNA base excision repair gene *XRCC1* is associated with several phenotypic markers of reduced DNA repair capacity. Results from several epidemiologic studies suggest that the *Arg399Gln* polymorphism may influence susceptibility to several cancers including bladder cancer; however, data from large population-based studies are lacking. In a population-based case-control study from New Hampshire, we observed a reduced risk among those homozygous for the *Arg399Gln XRCC1* variant polymorphism compared with those with one or two wild-type alleles (odds ratio 0.6, 95% confidence interval 0.4-1.0). There was no indication of a gene-environment interaction between cigarette smoking and the variant genotype. Our data are consistent with a potential role of the *XRCC1 Arg399Gln* polymorphism in bladder cancer susceptibility and further suggest that there may be DNA lesions important in bladder carcinogenesis, repaired by the base excision repair mechanism, that are not directly associated with tobacco smoking. (Cancer Epidemiol Biomarkers Prev 2004;13(8):1337–41)

Introduction

In addition to aryl amines, tobacco smoke contains compounds such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, catechol, and hydroquinone that generate free radicals and induce oxidative damage to DNA (1, 2). Consequently, constitutional variation in an individual's ability to repair DNA base damage may confer differential risk for smoking-related bladder cancer. Of potential importance is XRCC1, an abundant nuclear zinc finger protein that is part of a DNA binding protein complex. Although the XRCC1 protein has no enzymatic activity, it interacts with key enzymes involved in base excision repair including poly(ADP-ribose) polymerase (3), DNA polymerase β (3, 4), DNA ligase III, and AP endonuclease (APE1), a rate-limiting enzyme in base excision repair (5). Therefore, polymorphisms causing amino acid substitutions may impair the interaction of XRCC1 with the other enzymatic proteins and hence alter base excision repair activity.

Human *XRCC1* maps to chromosome 19q13.2 (6) and is composed of 17 exons (7). An exon 10 variant at codon 399 (8) leads to an Arg-to-Gln amino acid change and has been associated with several phenotypic alterations including higher levels of aflatoxin B1-DNA adducts, glycophorin A mutations (9), and polyphenol DNA adducts (10) in human tissues. Among current smokers, those with the homozygote variant genotype (*Gln/Gln*) had an increased frequency of sister chromatid exchanges in peripheral blood lymphocytes compared with the heterozygote or homozygote wild-type individuals (10). Several previous epidemiologic studies reported altered cancer risks associated with the *Arg399Gln XRCC1* polymorphism; in particular, two reports from hospital-based studies observed a lower prevalence of the homozygous variant genotype among bladder cancer cases than controls (11, 12), and a third hospital-based study (13) noted this trend among heavy smokers. However, a recent hospital-based study from Sweden found no association with the *XRCC1 Arg399Gln* genotype, but data were not consistently available on smoking history; thus, gene-environment interactions were not examined (14). Therefore, we investigated the possible role of the *Arg399Gln XRCC1* polymorphism and its potential interaction with cigarette smoking on the risks of bladder cancer in a population-based study.

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Materials and Methods

Study Population. We identified bladder cancer cases from the New Hampshire State Cancer Registry where an initial report of cancer is required within 15 days of diagnosis. This study includes incident cases of bladder cancer diagnosed between July 1, 1994 and March 31, 1998. Controls are randomly selected from drivers' license records (for those ages <65 years) and

from Medicare enrollment files (for those ages >65 years). All study participants are restricted by residency (New Hampshire), accessibility (having a telephone), language (English speaking), and age (25 to 74 years).

A total of 459 bladder cancer cases and 665 controls were interviewed; 85% of cases and 70% of controls were confirmed to be eligible for the study. Reasons for nonparticipation included physician's denial to contact their patient (10 cases); deceased (63 cases and 18 controls); no answer after 40 attempts distributed over days, evenings, and weekends (3 cases and 17 controls); refusal (75 cases and 261 controls); and mentally incompetent or too ill to take part (8 cases and 29 controls). We compared study participants and nonparticipants with regard to age, sex, and residence in an urban area. Both groups seem to be similar with regard to these factors. We shared a control group with a study of nonmelanoma skin cancer conducted covering a diagnostic period from July 1, 1993 to June 30, 1995 (15). We selected additional controls for bladder cancer cases diagnosed between July 1, 1995 and June 30, 1997 frequency matched to these cases on age (25 to 34, 35 to 44, 45 to 54, 55 to 64, 65 to 69, or 70 to 74 years) and gender.

Potential subjects were informed about the aims and requirements of the study, and informed consent for participation was obtained in accordance with institutional guidance at Dartmouth Medical School and Harvard School of Public Health. For >95% of participants, the interview was conducted in-person, usually at the subject's home; if a subject refused an in-person interview, it was conducted by telephone. Interviewers were masked to the study hypotheses and the case-control status of the participant. All interviewers underwent extensive training, and interviews were tape recorded for quality control purposes. Data were gathered regarding residence, occupational history, drinking water supply, demographic information, tobacco smoking, use of hair dyes, medical and family history of cancer, coffee consumption, and socioeconomic status.

A standardized histopathology review was conducted by the study pathologist, and from this review, we excluded 11 subjects who were initially reported to the cancer registry as having bladder cancer. Of the remaining cases, blood samples were available on 355 (79%) cases. A total of 558 (84%) controls provided a blood sample. Fourteen samples had missing data for *XRCC1* genotype due to PCR failure after duplicate attempts, leaving 355 cases and 544 controls for the analysis. Non-Hispanic Whites comprised 96.7% (n = 868) of the population studied; <1% of subjects reported their race/ethnicity as Hispanic (n = 5), Black (n = 1), American Indian (n = 7), or Asian (n = 1), and ~2% identified themselves as in the Other race/ethnicity category (n = 15) or refused to answer the question (n = 2).

***XRCC1* Genotyping.** Using the QIAamp Blood Kit (Qiagen, Hilden, Germany), we extracted genomic DNA from whole blood. *XRCC1* genotype was assayed using PCR followed by RFLP. The reaction for PCR was completed in a 50 μl reaction volume containing 0.3 ng DNA, 0.2 mmol/L of each primer (sense 5′-CCAAGTACAGCCAGGTCCTA-3′ and antisense 5′-AGTCTGACTCCCCTCCGGAT-3′), 1.25 units Taq DNA polymerase (Perkin-Elmer, Branchburg, NJ), 1.25 mmol/L deoxynucleotide triphosphates, 10 mmol/L Tris-HCl (pH 8.3, 25°C), 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, and 0.001% (w/v) gelatin. PCR cycle conditions were holding at 94°C for 5 minutes and 35 cycles of denaturation at 94°C, annealing at 60°C, and extension at 72°C for 1 minute. After amplification, PCR products were digested with *Msp*I (New England Biolabs, Beverly, MA) as described by the supplier. Each digested sample was electrophoresed in agarose gel in Tris-borate EDTA buffer. The gel was stained with ethidium bromide, visualized on a transilluminator under UV light, and photographed. For quality control purposes, laboratory personnel were blinded to case-control status, and both negative and positive controls (including every 10th sample as a masked duplicate) were included.

Statistical Analysis. Odds ratios (OR) and 95% confidence intervals (95% CI) were estimated using logistic regression with *XRCC1* genotype as the primary risk factor of interest. We classified *XRCC1* genotype as homozygous wild-type (*Arg/Arg*), heterozygous (*Arg/Gln*), and homozygous variant (*Gln/Gln*) with the *Arg/Arg* genotype as the reference group. Additionally, we computed the ORs for the homozygous variant genotype using one or two wild-type alleles as the reference group (*Arg/Arg* and *Arg/Gln*) to permit comparison with an earlier study (12). We tested the interaction between genotype and smoking status (never or ever) and number of pack-years smoked (never smoked, 1 to 34 pack-years, or ≥35 pack-years) using a likelihood ratio test comparing the models with and without interaction terms (16). We conducted these analyses for all bladder cancers combined and restricted to transitional cell carcinomas. We further did analyses of the association between *XRCC1* genotype and invasiveness (noninvasive versus invasive tumors) using polycotomous logistic regression, excluding *in situ* tumors (17). Lastly, we examined whether *XRCC1* genotype was especially associated with early age at onset of bladder cancer (~1 decade before the median, i.e., age <50 years) or later age at onset (age ≥50 years) using polycotomous logistic regression and computed the hazard ratio for *XRCC1* by age at bladder cancer diagnosis using a Cox proportional hazard model (18). We considered age (continuous in years), gender (female or male), highest level of education attained (high school or less, college, or graduate school), and first-degree family history of bladder cancer (yes or no) as potentially confounding factors in our analyses.

Results

Compared with the controls, cases were slightly older and more likely to be male (Table 1). Cases also were significantly more likely to be current or former smokers and have a greater number of pack-years smoked, less education, and a first-degree family history of bladder cancer (Table 1).

The variant *Arg399* allelic frequency among controls was 0.37, and the distribution of the polymorphism was tested for Hardy-Weinberg equilibrium (χ^2 = 4.66, 1 *df*, P = 0.03). The prevalence of the homozygote variant genotype was 16% for controls and 10% for cases (Table 2). The OR (95% CI) for bladder cancer among

Table 1. Selected characteristics of bladder cancer cases and controls*

	Cases (n = 355), n (%)	Controls (n = 544), n (%)	Age- and Sex-Adjusted OR (95% CI)
Mean (SD) age (y)	63 (9)	62 (10)	1.1 (1.0-1.3)† per decade
Gender			
Women	76 (21)	191 (35)	1.00 (reference)
Men	279 (79)	353 (65)	1.9 (1.4-2.6)‡
Smoking history			
Never	70 (20)	162 (30)	1.00 (reference)
Former	177 (50)	283 (52)	1.2 (0.9-1.8)
Current	108 (30)	99 (18)	2.4 (1.6-3.6)
Mean (SD) pack-years	48 (34)	32 (27)	1.2 (1.1-1.3) per 10 years
Education			
High school or less	209 (60)	270 (50)	1.00 (reference)
College	103 (29)	173 (32)	0.8 (0.6-1.0)
Graduate school	39 (11)	98 (18)	0.5 (0.3-0.8)
Family history of bladder cancer (first-degree relative)			
No	309 (94)	523 (99)	1.00 (reference)
Yes	21 (6)	6 (1)	6.7 (2.6-17.1)

*Excludes cases with missing data on pack-years (among ever smokers, 24 controls and 6 cases); education (3 controls and 4 cases); and family history of bladder cancer (15 controls and 25 cases).
†Adjusted for gender.
‡Adjusted for age.

homozygote variants was 0.8 (0.5-1.2; Table 2) compared with those who were homozygous wild-type and 0.6 (0.4-1.0; Table 2) compared with those with one or more wild-type allele. Adjustment for potentially confounding factors (i.e., family history or level of education) did not appreciably affect the ORs. We examined potential interaction between *XRCC1* genotype and smoking status (never versus ever) but found no evidence of an interaction (never smokers OR 0.7, 95% CI 0.3-1.6; ever smokers OR 0.6, 95% CI 0.4-1.0; P for interaction = 0.62; Table 2). We did not find any indication of an interaction when we examined smoking history as the number of pack-years smoked (P for interaction = 0.96; Table 2).

The inverse association between *Gln/Gln* genotype and bladder cancer risk was largely confined to individuals diagnosed at age ≥50 years (OR 0.6, 95% CI 0.3-0.9;

Table 2. Genotype frequencies and OR (95% CI) for *XRCC1* codon 399 genotype, smoking, and bladder cancer risk

Smoking	*XRCC1* Codon 399	Controls, n (%)	Cases, n (%)	Adjusted OR (95% CI)
All	*Arg/Arg*	228 (42)	132 (37)	1.00 (reference)
	Arg/Gln	230 (42)	187 (53)	1.4 (1.0-1.9)*
	Gln/Gln	86 (16)	36 (10)	0.8 (0.5-1.2)
	Arg/Arg + Arg/Gln	458 (84)	319 (90)	1.00 (reference)
	Gln/Gln	86 (16)	36 (10)	0.6 (0.4-1.0)*
Never	*Arg/Arg*	65 (40)	22 (31)	1.00 (reference)
	Arg/Gln	73 (45)	40 (57)	1.8 (0.9-3.3)†
	Gln/Gln	24 (15)	8 (11)	0.9 (0.4-2.4)
	Arg/Arg + Arg/Gln	138 (85)	62 (89)	1.00 (reference)
	Gln/Gln	24 (15)	8 (11)	0.7 (0.3-1.6)†
Ever	*Arg/Arg*	163 (43)	110 (39)	1.00 (reference)
	Arg/Gln	157 (41)	147 (52)	1.3 (0.9-1.9)*
	Gln/Gln	62 (16)	28 (10)	0.7 (0.4-1.2)
	Arg/Arg + Arg/Gln	320 (84)	257 (90)	1.00 (reference)
	Gln/Gln	62 (16)	28 (10)	0.6 (0.4-1.0)*
Pack-years smoked				
>34	*Arg/Arg + Arg/Gln*	127 (24)	161 (46)	1.00 (reference)
>34	*Gln/Gln*	21 (4)	16 (5)	0.6 (0.3-1.3)†
1-34	*Arg/Arg + Arg/Gln*	173 (33)	90 (26)	0.8 (0.5-1.4)
1-34	*Gln/Gln*	37 (7)	12 (3)	0.5 (0.2-1.1)
None	*Arg/Arg + Arg/Gln*	138 (27)	62 (18)	0.9 (0.5-1.8)
None	*Gln/Gln*	24 (5)	8 (2)	0.6 (0.2-1.8)

*Adjusted for age, gender, and pack-years.
†Adjusted for age and gender.

Table 3. *XRCC1* Codon 399 in relation to age at diagnosis and invasiveness of bladder cancer

	Arg/Arg + Arg/Gln, *n* (%)	OR* (95% CI)	*Gln/Gln*, *n* (%)	OR* (95% CI)
Age at diagnosis of bladder cancer (y)				
Age at diagnosis ≥50 y	294 (91)	1.00 (reference)	29 (9)	0.6 (0.3-0.9)†
Age at diagnosis < 50 y	25 (78)	1.00 (reference)	7 (22)	1.5 (0.6-3.6)
Controls	458 (84)		86 (16)	
Ratio of the OR* (95% CI)		1.00 (reference)		0.4 (0.1-0.9)†
Invasiveness of bladder cancer				
Invasive	77 (88)	1.00 (reference)	11 (12)	0.8 (0.4-1.7)
Noninvasive	234 (91)	1.00 (reference)	22 (9)	0.5 (0.3-0.9)†
Controls	458 (84)		86 (16)	
Ratio of the OR* (95% CI)		1.00 (reference)		1.6 (0.7-3.5)

*Adjusted for gender and pack-years using polycotomous logistic regression.
†$P < 0.05$.

Table 3); however, the hazard ratio for age at diagnosis did not differ by *XRCC1* genotype (hazard ratio 1.1, 95% CI 0.8-1.6 for *Gln/Gln* versus *Arg/Arg* and *Arg/Gln*). There did not seem to be any significant differences in the ORs by tumor type (e.g., invasive or noninvasive; Table 3). In addition, we did not detect differences in the ORs when we restricted the analysis to transitional cell carcinomas (data not shown).

Discussion

We found that the homozygote variant genotype of *XRCC1* was inversely associated with bladder cancer risk and observed no evidence of an interaction between smoking and the *XRCC1* polymorphism. Findings from our population-based study are strikingly similar to the two prior studies of the *XRCC1 Arg399Gln* polymorphism in which there was a lower prevalence of the *Gln/Gln* genotype among bladder cancer cases than controls (11, 12). As in our study, these studies found no clear evidence of an interaction between exposure to tobacco smoke and the variant genotype on bladder cancer risk. However, an additional hospital-based study found a reduced risk of bladder cancer associated with the *Arg399* polymorphism only among heavy smokers (13), and in another hospital-based study, no association was observed, although smoking history was not examined (14).

Studies of the phenotype of the *Arg399Gln* variant suggest that the *Gln399* allele results in higher levels of DNA adducts (10), mutations (9), and sister chromatid exchanges (10) and, in theory, could lead to an increased incidence of malignancy. However, the reduced cancer risk found in ours and other studies is consistent with the hypothesis that the variant XRCC1 protein, and its consequent diminished DNA repair, could enhance damage-related apoptosis in individual cells (19). Seedhouse et al. (20) investigated the association of *XRCC1* polymorphisms on secondary (treatment-related) leukemia and observed a similar inverse association of cancer risk with the *XRCC1* polymorphism, citing the enhancement of apoptosis as a possible mechanism. In this case, therapy results in dramatically enhanced DNA damage to surrounding normal cells, giving rise to high secondary cancer risks. The *XRCC1* homozygous variant genotype was most strongly associated with protection from secondary leukemia (OR 0.28, 95% CI 0.09-0.88), with greater protection for therapy-associated disease than for primary disease (OR 0.57, 95% CI 0.3-1.08). In the study of Duell et al. (10), there was evidence that the association of DNA adduct formation and the *Gln/Gln* genotype increased with age. These results support our finding that the inverse relationship with the variant genotype was present largely among those diagnosed at age ≥50 years. However, using a survival analytic approach, there was no evidence that the association varied by age at diagnosis, making our findings inconclusive.

The absence of a detectable interaction with tobacco smoking is somewhat surprising. If true, it implies that the phenotypically relevant DNA damage that serves as the essential substrate for the *XRCC1 Gln* allele is not more prevalent in smokers (given that the kinetics of DNA repair are stable and approximately linear) and thus may be due to other factors. The exposure and damage of cellular DNA as well as the DNA damage response are complex phenomena, and our data indicate that there is a need to more fully understand the relationship among polymorphic DNA repair genes, carcinogen exposure, and cancer risk.

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