

XPD Codon 751 Polymorphism, Metabolism Genes, Smoking, and Bladder Cancer Risk

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Abstract

Cigarette smoking is the main risk factor for bladder cancer, accounting for at least 50% of bladder cancer in men. Cigarette smoke is a rich source of arylamines, which are detoxified by the NAT2 enzyme and activated by the NAT1 enzyme to highly reactive species that can form bulky adducts on DNA. DNA damage from such adducts is mainly repaired by the nucleotide excision repair pathway, in which the XPD protein functions in opening the DNA helix. We hypothesized that an XPD codon 751 polymorphism (Lys-to-Gln amino acid change) could affect the repair of smoking-induced DNA damage and could be associated with bladder-cancer risk. We also hypothesized that allelic variants of the NAT1 and NAT2 genes might modify the effect of the XPD codon 751 polymorphism on smoking-associated bladder-cancer risk. We determined the XPD codon 751 genotype for 228 bladder-cancer cases and 210 controls who were frequency-matched to cases by age, sex, and ethnicity, and we used our previously published data on the NAT1 and NAT2 genotypes for these same individuals (J. A. Taylor *et al.*, *Cancer Res.*, 58: 3603–3610, 1998). We found a slight decrease in risk for the XPD codon 751 Gln/Gln genotype (adjusted odds ratio: 0.8; 95% confidence interval: 0.4–1.3) compared with subjects with the Lys/Lys or Lys/Gln genotypes. The analysis with smoking showed that smokers with the Lys/Lys or Lys/Gln genotypes were twice as likely to have bladder cancer than smokers with the Gln/Gln genotype (test of interaction $P = 0.03$). The combined presence of the NAT1/NAT2 high-risk genotype and the XPD Lys/Lys or Lys/Gln genotypes ignoring smoking had an odds ratio that was only slightly higher than expected, assuming no genotype-genotype interaction ($P = 0.52$). We found little evidence for a gene-gene-exposure, three-way interaction

among the XPD codon 751 genotype, smoking, and the NAT1/NAT2 genotype.

Introduction

Cigarette smoking is the primary risk factor for bladder cancer, increasing risk up to 4-fold (1–5). Cigarette smoke contains a wide variety of chemical carcinogens, including polycyclic aromatic hydrocarbons, aromatic amines, and N-nitroso compounds, that can form bulky adducts on DNA of urothelial cells after activation by specific metabolism enzymes (6). Other metabolism enzymes prevent the conversion of procarcinogens into reactive species; therefore, the balance between activation and detoxification of carcinogens, among other biological processes, affects the amount of DNA damage that accumulates in cells.

Arylamines are of particular interest because they are present in cigarette smoke and occupational exposures and have been shown to raise the risk of bladder cancer up to 100-fold (7). Arylamines are metabolized in the liver where they can be N-acetylated to less reactive forms by NAT2. Alternatively, they can be N-hydroxylated by CYP1A2 and ultimately serve as a substrate in bladder epithelium for O-acetylation by NAT1 forming highly reactive species that create bulky DNA adducts (8). The NAT2 and NAT1 genes are polymorphic, and these polymorphisms are associated with differences in enzymatic activity and carcinogen metabolism (9). We (5) and others (10) have demonstrated previously, that the NAT2-slow alleles and the NAT1*10 allele are risk factors for smoking-associated bladder cancer (reviewed in Ref. 11). Our study has also shown evidence of a NAT1-NAT2-smoking interaction in bladder-cancer risk (5).

Bulky adducts on DNA, like those formed by arylamines, are repaired mostly by the NER³ pathway. Several epidemiological studies have used *in vitro* assays to test the ability of individuals to repair DNA damage induced by different carcinogens, such as BPDE and UV radiation, that require the NER pathway, (reviewed in Ref. 12). These studies found that individuals vary in their abilities to repair DNA damage and that cancer patients tend to have lower DNA-repair capacity when compared with healthy controls (reviewed in Ref. 12). Therefore, polymorphic NER genes are reasonable candidates as susceptibility genes.

In this study, we explored the role of the XPD gene in bladder cancer risk. The XPD protein is a DNA-dependent ATPase/helicase (13) that is associated with the TFIIH transcription-factor complex, reviewed in Ref. 14, and plays a role in NER (15), basal transcription, and apoptosis (16). During transcription the main function of XPD is to maintain the stability of the TFIIH transcription-factor complex. During

Received 10/5/01; revised 6/3/02; accepted 6/14/02.

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³ The abbreviations used are: NER, nucleotide excision repair; BPDE, benzo(a)pyrene diol epoxide; OR, odds ratio; CI, confidence interval.

NER, XPD participates in the opening of the DNA helix to allow the excision of the DNA fragment containing the damaged base, (reviewed in Ref. 14). There are four described polymorphisms that induce amino acid changes in the protein (17): in codons 199 (Ile to Met), 201 (His to Tyr), 312 (Asp to Asn) and 751 (Lys to Gln). Several studies have analyzed possible associations between some of these polymorphisms and different phenotypic endpoints with different results. Two studies found evidence that suggests that the codon 751 Gln allele may provide a greater protection against DNA damage. The phenotypic endpoints of these studies were chromatid aberrations (18), and double-strand breaks after UVC exposure (19). Conversely, one study of lung patients found evidence that suggests that the codon 312 Asn allele and the codon 751 allele may provide a diminished protection against DNA damage. The phenotypic endpoint of this study was BPDE damage repair measured by the host-cell reactivation assay, (20). A third study found no associations between the codon 751 polymorphism and the presence of polyphenol DNA adducts or sister chromatid exchange (21). More recently, Seker *et al.* (22) were able to show, using constructs that carry the different variants for the codon 312 and 751 polymorphisms, that the codon 312 Asn allele had a higher apoptotic response after UV exposure. No differences in apoptotic response were observed for the codon 751 Gln allele. However, they were able to show that the codon 751 allele is within the p53 binding domain. Seker *et al.* did not find differences in p53 binding between the codon 751 Lys or Gln allele; however, the authors suggest that this could be attributable to the sensitivity of the assay they used and/or the fact that the two alleles have minor effects on p53 binding.

We examined the role of the XPD codon 751 polymorphism in a bladder cancer case-control study using cigarette smoking as a surrogate measure for arylamine exposure. We have also explored the combined effect of the XPD and NAT1 and NAT2 genotypes on bladder cancer risk.

Materials and Methods

Subjects. Bladder-cancer patients ($n = 228$) and control individuals ($n = 210$) were recruited from the urology clinics at Duke University Medical Center and the University of North Carolina Hospitals as described previously (5, 23). Cases were urology clinic patients with histologically confirmed transitional cell carcinoma. Controls were urology patients without a history of cancer from the same clinics. Among male controls, the most frequent diagnoses were benign prostate hypertrophy and impotence, and among female controls, the most frequent diagnosis was urinary incontinence. Controls were frequency matched to cases based on ethnicity, sex, and age at interview (a 10-year interval). Only three individuals belonged to an ethnic group other than black or white, one case (double-racial heritage) and two controls (native American and native Alaskan). Given their low number, we did not include these three subjects in our analyses. All individuals were administered a structured questionnaire that detailed their medical history, including detailed smoking history. 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 Tris-EDTA buffer (10 mM Tris, 1 mM EDTA), and frozen until use. The polymorphism in XPD codon 751 was analyzed as described previously (18). Briefly, the fragment surrounding codon 751 was amplified by PCR with 50 ng of

DNA in a final volume of 15 μ l containing 1 \times PCR buffer (Perkin-Elmer, Foster City, CA), 0.2 mM deoxynucleotide triphosphate, 2 mM MgCl₂, 0.8 μ M of each primer, and 0.5 units of AmpliTaq Gold (Perkin-Elmer). PCR reactions were carried out in a PE 9700 thermocycler (Perkin-Elmer) 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. These PCR products were digested with MboII, resolved in 2% 3:1 NuSieve agarose gels (FMC Bioproducts, Rockland, ME), and stained with ethidium bromide. The polymorphisms in NAT1 and NAT2 were determined in our previous study (5). Briefly, a PCR-RFLP and an allele specific PCR method were used to detect several common polymorphisms in NAT1 and NAT2 that have been associated previously with altered function or risk. For NAT1, these included: A1088T, C1095A, 9-nt deletion (nt 1064–1091). The allele containing both A1088T and C1095A is referred to as NAT1*10. For NAT2, the following single-nucleotide polymorphisms were genotyped: G191A, C341T, G590A, and G857A. These changes identify the four most common NAT2-slow alleles (NAT2*5, NAT2*6, NAT2*7, NAT2*14).

Statistical Analysis. We used standard methods for $2 \times k$ contingency tables to analyze categorical variables without adjustment for covariates (24). 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 (25). We used standard logistic regression methods (26) when adjusting for age, sex, or ethnicity and when examining interactions between the polymorphism and smoking. We tested for interaction on a multiplicative scale. Given the few blacks in our study, we did not analyze them as a separate group, but included them in combined analyses with whites while including ethnicity as a covariate. For gene-environment-interaction analyses, we used categorized versions of ever or never smoking and years of smoking as measures of smoking exposure. In addition, we used years of smoking as a continuous variable because we had observed previously that this variable was more predictive of bladder-cancer risk in our data (5). To examine the joint effects of exposure to cigarette smoking expressed as a continuous variable and the XPD genotype or the XPD and NAT1/NAT2 genotypes, we used an approach that we have used previously (5, 23), which consists of fitting a series of logistic regression models and comparing them using likelihood-ratio tests to test relevant hypotheses. We assumed that the dose-response for exposure to smoking was linear in the log-odds scale. Within each model, differences between slopes represent genotype-exposure interactions and differences between intercepts represent genotype effects among nonsmokers. To examine the joint effect of the XPD and NAT1/NAT2 genotypes, we used a dichotomous variable for the combined presence or absence of the risk allele for NAT1 (NAT1*10) and the risk "slow" alleles for NAT2 (NAT2*5, NAT2*6, NAT2*7 and NAT2*14). We have reported previously that the associations between the NAT1 and NAT2 genotypes and bladder cancer can be adequately estimated using a dichotomous variable for genotype: a high-risk group comprised subjects carrying at least one copy of the NAT1*10 allele along with the two NAT2-slow alleles, and a lower risk group comprised all other NAT1 and NAT2 genotype combinations (5). We examined smoking, XPD, and NAT1/NAT2 jointly using four genotypes defined by cross-classifying dichotomous XPD and NAT1/NAT2 combined genotypes. All tests were two-tailed. All analyses were performed using the statistical packages Egret (Cytel Software Corporation, Cam-

Table 1 Demographic information

	Cases (n = 233)			Controls (n = 209)			OR (95% CI) ^a	P ^b
	n	%	Mean (SD)	n	%	Mean (SD)		
Sex								0.24
Female	53	22.8		38	18.2		ND ^c	
Male	180	77.2		171	81.8			
Ethnicity								0.32
White	214	91.8		197	94.3		ND	
Black	19	8.2		12	5.7			
Age range (years)								0.10
≤60	59	25.3		65	31.1			
60–70	90	38.6		88	42.1		ND	
>70	84	36.1		56	26.8			
Mean Age			65.8 (10.8)			63.4 (10.3)		
Smoking History								<0.001
Never	38	16.4		75	35.9		1.0 ^{ref}	
Quit	136	58.6		108	51.7		3.2 (1.9–5.3)	
Current	58	25.0		26	12.4		6.1 (3.2–11.7)	
Pack-years								<0.001
Nonsmoker	42	18.1		78	37.5		1.0 ^{ref}	
1–35 pack-years	75	32.3		76	36.5		2.5 (1.4–4.3)	
>35 pack-years	115	49.6		54	26.0		5.6 (3.2–9.9)	
Mean pack-years			39.9 (35.7)			24.2 (32.3)		
Years smoked								<0.001
Nonsmoker	38	16.4		75	35.9		1.0 ^{ref}	
1–34 years smoked	91	39.4		92	44.0		2.6 (1.5–4.5)	
>34 years smoked	102	44.2		42	20.1		5.9 (3.3–10.5)	
Mean years smoked			29.3 (19.8)			16.8 (16.8)		

^a Adjusted for age, sex, and ethnicity.

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

^c ND, not determined; cases and controls were frequency-matched on these variables.

bridge, MA) and Stata version 6 (Stata Corporation, College Station, TX).

Results

Cases and controls were similar for the frequency-matched variables sex, ethnicity, and age at interview (Table 1). Cases were more likely to ever have smoked than controls and, on average, smoked 12.7 years longer and 15.9 pack-years more than controls (OR = 6.1; 95% CI = 3.2–11.7). Unless otherwise noted, all results presented below are for whites and blacks combined, adjusted for age, sex, and ethnicity.

XPD Codon 751 Polymorphism and Bladder-Cancer Risk.

Among whites, the frequency of the codon 751 Gln allele was 0.38 and 0.36 for cases and controls, respectively. Among blacks, the corresponding frequencies were 0.35 and 0.22, respectively. There were no statistically significant differences between the observed genotypic frequencies among controls and those expected under the Hardy-Weinberg law ($P = 0.50$ for whites and $P = 0.49$ for blacks). Because we did not observe an association between the Lys/Gln genotype and bladder cancer, we combined these subjects with those who had the Lys/Lys genotype and compared this combined category to subjects with the Gln/Gln genotype, making the *XPD* genotype a dichotomous variable. Compared with individuals homozygous for the Lys allele, the OR for bladder cancer for subjects with one copy of the Gln allele was 1.0 (95% CI = 0.7–1.5) and 0.8 (95% CI = 0.4–1.4) for those with two copies of the Gln allele (Table 2). Given the lack of association we observed between the Lys/Gln genotype and bladder-cancer risk, for the remainder of the study we used as the reference group subjects with one or two copies of the Lys allele.

***XPD* Codon 751 Polymorphism and Smoking.** We investigated whether the *XPD* genotype could modify the association between exposure to cigarette smoking and bladder cancer (Table 3). For these analyses we use as a reference group subjects who were nonsmokers and carried the more frequent *XPD* allele, that is, the Lys allele. We observed that among never smokers the Gln/Gln genotype was more strongly associated with bladder cancer than the Lys/Gln or Lys/Lys genotypes combined (OR = 2.2, 95% CI = 0.7–6.4), although the number of subjects in this category is small. However, ever smokers with the Gln/Gln were half as likely to have bladder cancer than ever smokers with the Lys/Lys or Lys/Gln genotype (OR for the Gln/Gln genotype within this smoking stratum = 0.5; 95% CI = 0.3–1.0). The OR for the combined presence of the Gln/Gln genotype and ever smoking (observed OR = 2.4) was lower than the expected when assuming no interaction on the multiplicative scale (expected OR = 10.3). We carried out the same analysis using years of smoking as a categorical variable (Table 3). Among both moderate smokers (1–34 years) and heavy smokers (>34 years), we observed that subjects with the Gln/Gln genotype had lower ORs than those with the Lys/Lys or Lys/Gln genotypes (Table 3). Within each smoking strata, we observed a lower risk associated with the Gln/Gln genotype, with an OR = 0.6 (95% CI = 0.2–1.4) for subjects who smoked 1–34 years and an OR = 0.5 (95% CI = 0.2–1.4) for those who smoked >34 years. Tests for *XPD*-smoking interaction were statistically significant when we classified smoking as a dichotomous variable (ever/never) for whites only and blacks and whites combined (Table 3). When we classified smoking into three levels of years smoked, tests of *XPD*-smoking interaction were of borderline statistical significance

Table 2 XPD codon 751 polymorphism genotypic frequencies and bladder cancer risk

Genotype	Whites				Blacks		Whites and blacks combined	
	Controls (%)	Cases (%)	OR _{adj} ^a	95% CI	Controls (%)	Cases (%)	OR _{adj} ^b	95% CI
Codon 751								
Lys/Lys	79 (40)	84 (40)	1.0 ^{ref}		5 (38)	11 (58)	1.0 ^{ref}	
Lys/Gln	88 (45)	99 (47)	1.1	0.7–1.6	7 (54)	6 (32)	1.0	0.7–1.5
Gln/Gln	30 (15)	27 (13)	0.8	0.4–1.5	1 (8)	2 (11)	0.8	0.4–1.4
Lys/Lys + Lys/Gln	167 (85)	183 (87)	1.0 ^{ref}		12 (92)	17 (90)	1.0 ^{ref}	
Gln/Gln	30 (15)	27 (13)	0.8	0.4–1.4	1 (8)	2 (10)	0.8	0.4–1.4
Total	197	210			13	19		

^a Adjusted for age, sex.^b Adjusted for age, sex, and ethnicity.

Table 3 XPD codon 751 polymorphism and smoking, combined analysis

Smoking	XPD codon 751	Whites				Test for interaction <i>P</i>	Blacks		Whites and blacks combined		
		Cases	Controls	OR _{adj} ^a	95% CI		Cases	Controls	OR _{adj} ^b	95% CI	Test for interaction <i>P</i>
Status											
Never	Lys/Lys + Lys/Gln	27	64	1.0 ^{ref}			2	4	1.0 ^{ref}		
Never	Gln/Gln	9	8	2.2	0.7–6.4		0	0	2.2	0.7–6.4	
Ever	Lys/Lys + Lys/Gln	156	103	4.6	2.6–8.1		15	8	4.7	2.7–8.1	
Ever	Gln/Gln	18	22	2.5	1.1–5.5	0.03	3	1	2.4	1.1–5.3	
Years Smoked											
0	Lys/Lys + Lys/Gln	27	64	1.0 ^{ref}			2	4	1.0 ^{ref}		
0	Gln/Gln	9	8	2.3	0.8–6.8		0	0	2.3	0.8–6.7	
1–34	Lys/Lys + Lys/Gln	69	69	3.1	1.7–5.7		9	6	3.2	1.8–5.8	
1–34	Gln/Gln	10	16	1.9	0.7–4.9	0.06	0	0	1.9	0.8–4.9	
>35	Lys/Lys + Lys/Gln	87	34	7.4	3.9–14		5	2	7.2	3.9–14	
>35	Gln/Gln	8	6	4.0	1.2–13	0.07	1	1	3.6	1.2–11	

^a Adjusted for age and sex.^b Adjusted for age, sex, and ethnicity.

for whites only and statistically significant among blacks and whites combined (Table 3).

We also analyzed the XPD genotype and smoking using years of smoking as a continuous variable. We fitted separate lines for each XPD genotype, allowing separate gene main effects among nonsmokers, given by the intercepts, and separate smoking-dose response, given by the slopes; we refer to this as the “full model” (Fig. 1A). We found borderline statistically significant differences between this full model and a single-line model that assumed no differences in gene effects or gene-smoking interaction between the two genotypes ($\chi^2_{2df} = 4.3$; $P = 0.12$). To test for interaction, we compared the full model to a model that assumed no interaction by constraining both lines to have the same slope but allowing different intercepts. We found significant differences between these two models ($\chi^2_{1df} = 3.9$; $P = 0.05$), indicating that the model that included a multiplicative interaction term fit the data significantly better, and therefore supporting an XPD-smoking interaction (Fig. 1B). Given our finding that the Gln allele was associated positively with bladder cancer among nonsmokers, but inversely associated among smokers, we evaluated whether a model that did not allow for a gene effect among nonsmokers would still fit the data as well as the “full model.” Therefore, we fitted a model that allowed different smoking-dose response for each genotype, but where intercepts were constrained to be the same (Fig. 1C). We found no statistically significant differences between the full model and this simpler model ($\chi^2_{1df} = 1.2$;

$P = 0.26$). Using this simpler model, we again tested for gene-smoking interaction by comparing it to a model that fit both genotypes to a single line. This comparison showed that the fit of the one-line model was relatively poor ($\chi^2_{1df} = 3.01$; $P = 0.08$), suggesting that XPD-smoking interaction is not dependent on the association between the Gln allele and bladder cancer among nonsmokers.

We used a third approach to explore a possible XPD-smoking interaction by performing a case-only analysis. We compared the frequency of smoking among cases with the Lys/Lys or Lys/Gln genotypes to the frequency of smoking among cases with the Gln/Gln genotype. This approach allowed us to test for gene-smoking interaction without the potential bias introduced by differences in genotype frequency in different smoking strata among controls. Assuming that genotype and smoking are independent, the resulting ORs provide a direct measure of interaction on a multiplicative scale that is more precise than that obtained through case-control studies; this difference in precision is attributable to the lack of variance associated with controls (27, 28). We found that with increasing years of smoking, cases were more likely to have the Lys/Lys or Lys/Gln genotype, rather than the Gln/Gln genotype (1–34 years of smoking: OR = 1.8, 95% CI = 0.6–5.6; >34 years of smoking: OR = 2.6, 95% CI = 0.9–7.6). We found similar results when we used categorical variables for smoking status and pack-years. Therefore, our findings support an XPD-smoking interaction.

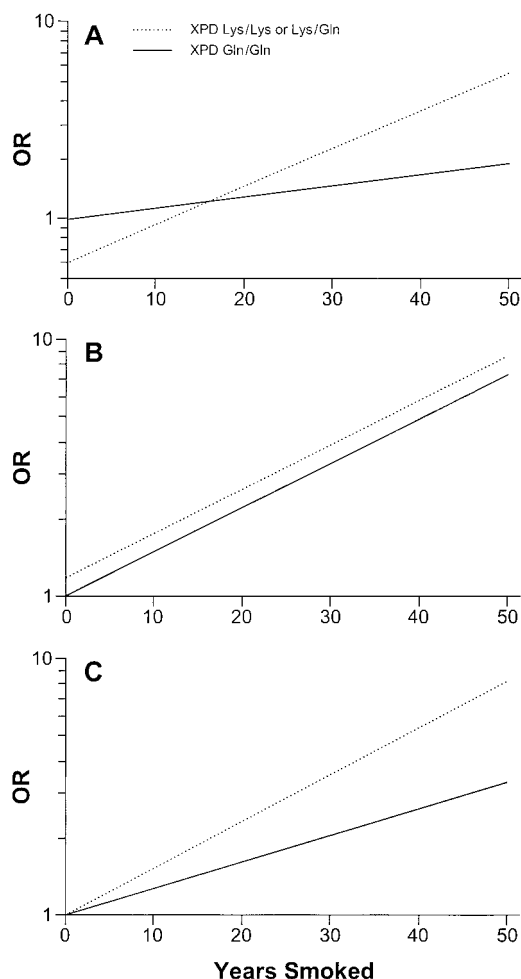


Fig. 1. ORs for the *XPD* codon 751 Lys/Lys-Lys/Gln or Gln/Gln genotypes plotted against years of smoking using different models. A, the full model, which allows separate gene effects among nonsmokers (intercepts) and separate smoking-dose response effects (slopes). B, simplified model that assumes no interaction by constraining both lines to a same slope, but allowing different intercepts. There were significant differences between models A and B ($\chi^2_{1df} = 3.9$; $P = 0.05$), suggesting an *XPD*-smoking interaction. C, model that allows different smoking-dose response for each genotype, but constrains intercepts to be the same, not allowing for gene effect among nonsmokers. There were no statistically significant differences between models A and C ($\chi^2_{1df} = 1.2$; $P = 0.26$).

***XPD* Codon 751, *NAT1*, and *NAT2* Polymorphisms.** To investigate whether the *XPD*-genotype association with bladder cancer could be modified by the status of metabolism enzymes, we performed a combined analysis of the *XPD* codon 751 polymorphism with data we had obtained previously on *NAT1* and *NAT2* polymorphisms for the same subjects (5). This earlier study suggested a three-way interaction between the *NAT1* and *NAT2* polymorphisms and exposure to cigarette smoke. Individuals with the combined genotype of one or more *NAT1**10 alleles and two *NAT2*-slow alleles, designated “*NAT1**10/*NAT2*-slow,” had a higher risk of bladder cancer with increasing years of smoking than did individuals who carried any of the other genotypic combinations (5). We analyzed the effect of the *XPD* codon 751 polymorphism in the presence or absence of the *NAT1**10/*NAT2*-slow high-risk genotype. For this analysis, we used as a reference those individuals at the lowest risk: those who lacked the *NAT1**10/*NAT2*-slow genotype and car-

ried the *XPD* codon 751 Gln/Gln genotype (Table 4). The combined presence of both *NAT1**10/*NAT2*-slow and *XPD* Lys/Lys or Lys/Gln risk genotypes was associated with an OR that was only slightly higher than what was expected under the assumption of no interaction on a multiplicative or additive scale (test of interaction $P = 0.52$).

***XPD*, *NAT1*, *NAT2*, and Smoking.** We analyzed whether cigarette smoking could modify the combined effects of the *NAT1*/*NAT2* and the *XPD* codon 751 genotype. We used years of smoking as a continuous variable using logistic regression models. We first fitted separate lines for each of the four genotypic combinations, allowing separate gene main effects among nonsmokers (intercepts) and separate smoking-dose response effects (slopes); we refer to this as the “full model” (Fig. 2A). We next tested whether the effect of smoking on bladder-cancer risk for each *XPD* genotype was the same in the presence of the *NAT1*/*NAT2* combined low- or high-risk genotypes. We fitted a model that assumed no three-way interaction and where the difference in slope between *XPD* Lys/Lys or Lys/Gln and *XPD* Gln/Gln is constrained to be the same whether the *NAT1**10/*NAT2*-slow is present or absent (Fig. 2B). We found no statistically significant differences between this model and the full model ($\chi^2_{2df} = 0.27$; $P = 0.87$), which provides little support for a three-way interaction between *XPD*, the combined *NAT1**10/*NAT2*-slow genotype, and smoking. Our previous study suggested that the *NAT1* and *NAT2* polymorphisms may not have an effect among nonsmokers (5); therefore, we next evaluated whether a model that did not allow for a gene effect among nonsmokers would still fit the data as well as the full model. We fitted a simplified model where all genotypes had a common intercept but were allowed different slopes (Fig. 2C). We found no statistically significant differences between the full model and the common intercept model ($\chi^2_{3df} = 1.49$; $P = 0.68$), leading us to adopt the latter model. We again tested for three-way interaction, by comparing this common intercept model to another one that assumed no three-way interaction and constrained the difference in slope between *XPD* Lys/Lys or Lys/Gln and *XPD* Gln/Gln to be the same regardless of the *NAT1*/*NAT2* genotype (Fig. 2D). We found no statistically significant differences between these two models ($\chi^2_{1df} = 0.10$; $P = 0.75$), again providing little support for a three-way interaction between *XPD*, the combined *NAT1**10/*NAT2*-slow genotype, and smoking.

***XPD* Codon 751 Genotype and Disease Prognosis Variables.**

A comparison between cases with and without the codon 751 Gln/Gln genotype showed that although the frequency of the Gln/Gln genotype among grade 1 tumors was slightly lower (4%) than that observed among grade 2 (9%), 3 (9%) or 4 (5%), the overall differences were not statistically significant ($P = 0.41$). Similarly, although the frequency of the Gln/Gln genotype among cases with ages at diagnosis less than the median (63 years old) was slightly lower than that among cases with ages at diagnosis higher than the median (13% versus 16%), this difference was not statistically significant ($P = 0.67$).

Discussion

The codon 751 polymorphism is located in the COOH-terminal domain of *XPD*. This region of the protein is essential for interaction with the p44 subunit of TFIIF, which stimulates *XPD* by increasing its helicase activity (29, 30). Both xeroderma pigmentosum and trichothiodystrophy patients who carry mutations in this region (*i.e.*, XPDR722W, XPDR716-730) have reduced *XPD* DNA helicase activity in TFIIF, which

Table 4 XPD codon 751 polymorphism and NAT1/NAT2 polymorphisms, combined analysis

NAT1/NAT2 risk genotype ^a	XPD codon 751 genotype	Whites				Test for interaction <i>P</i>	Blacks		Whites and blacks combined		
		Cases	Controls	OR _{adj} ^b	95% CI		Cases	Controls	OR _{adj} ^c	95% CI	Test for interaction <i>P</i>
–	Gln/Gln	23	25	1.0 ^{ref}			1	1	1.0 ^{ref}		
–	Lys/Lys + Lys/Gln	145	141	1.2	0.6–2.2		9	9	1.2	0.6–2.2	
+	Gln/Gln	4	4	1.1	0.2–5.0		0	0	1.1	0.2–5.0	
+	Lys/Lys + Lys/Gln	36	18	2.3	1.0–5.2	0.49	3	2	2.2	1.0–4.9	0.52

^a NAT1*10/NAT2-slow combined genotype.

^b Adjusted for age and sex.

^c Adjusted for age, sex, and ethnicity.

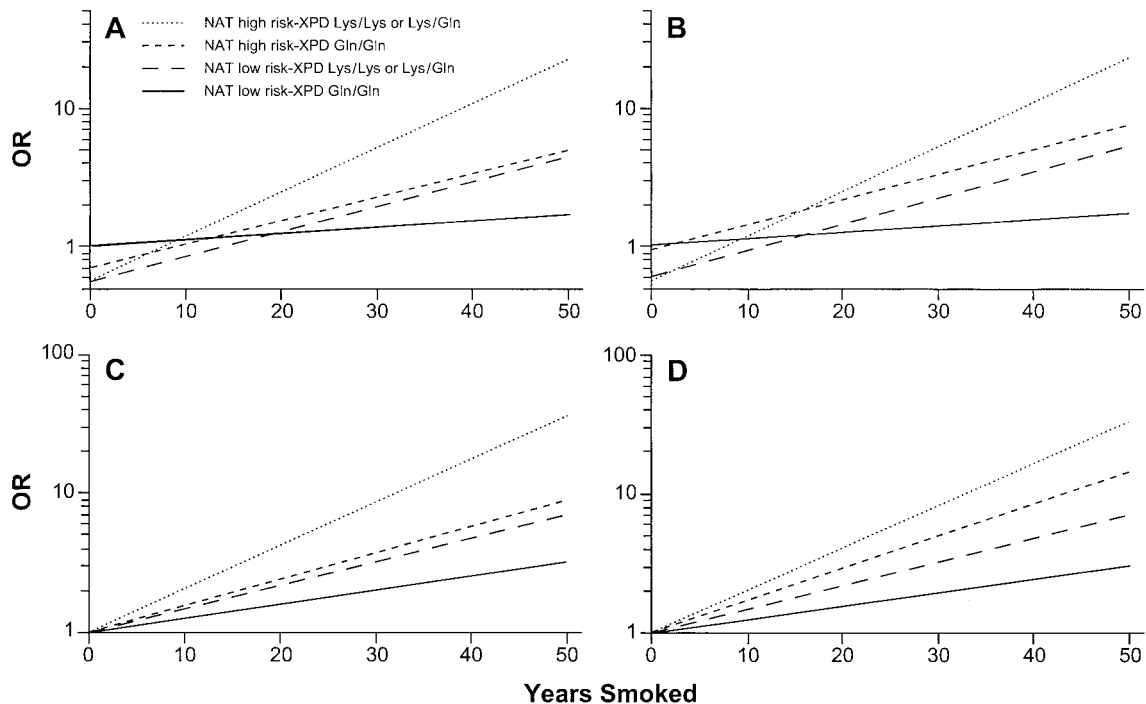


Fig. 2. ORs for four possible combinations of NAT1/NAT2 and XPD codon 751 genotypes plotted against years of smoking using different models. A, full model in which slope and intercept are fitted separately for each line, allowing separate gene effects among nonsmokers and smoking-dose response effects. B, model in which the difference in slope between the different XPD genotypes is forced to be the same for each NAT1/NAT2 genotype, while allowing different intercepts. There were no statistically significant differences between models A and B ($\chi^2_{1df} = 0.27$; $P = 0.87$), suggesting no three-way interaction among XPD, the combined NAT1*10/NAT2-slow genotype, and smoking. C, simplified model in which all lines are constrained to a common intercept, but are allowed separate slopes. There were no statistically significant differences between models A and C ($\chi^2_{3df} = 1.49$; $P = 0.68$), leading us to adopt model C. D, model in which all lines are constrained to a common intercept and the difference in slope between the different XPD genotypes is forced to be the same for each NAT1/NAT2 genotype. There were no statistically significant differences between models C and D ($\chi^2_{1df} = 0.10$; $P = 0.75$), suggesting no three-way interaction among XPD, the combined NAT1*10/NAT2-slow genotype, and smoking.

may account for the observed defects in NER observed in these patients (31).

Although the XPD codon 751 polymorphism appeared to have little effect on bladder-cancer risk when we ignored smoking, there was evidence of gene-smoking interaction. Regardless of whether smoking was treated as a categorical or continuous variable, the Gln allele appeared to have a positive association with bladder cancer among nonsmokers, but an inverse association with bladder cancer among smokers. Given the few nonsmoking cases and controls who were homozygous for the Gln allele, the point estimate for the nonsmokers is imprecise. If smoking and XPD genotype are independent among controls, the frequency of Gln homozygotes should be similar in nonsmokers (11%), 1–34-years smokers (19%), and >35-years smokers (15%). The variation in Gln-homozygote

frequency among controls is not surprising given the small sample size of these strata. However, the low frequency of Gln homozygotes among nonsmoking controls could be responsible partially for the positive association between Gln homozygotes and risk among nonsmokers and could be responsible for the interaction in the analyses using smoking as a categorical variable.

We were able to address this issue by performing a case-only analysis to estimate the effect of smoking using categorical variables. By excluding the controls from our analyses, we were able to test for a gene-smoking interaction more precisely because of the lack of potential bias introduced by slight differences in genotype frequencies in different smoking strata among controls and the lack of variance associated with controls (27, 28). Our analyses suggested that the observed XPD-

smoking interaction was not being driven by the nonsmoking controls because when we eliminated the control group, we still found support for the hypothesis of an *XPD*-smoking interaction. It is important to note that case-only analyses of interaction depend on the assumption that exposure and genotype are independent. Although this assumption appears reasonable in the case of *XPD* and smoking, small violations of this assumption can bias heavily estimates of interaction (32). Although we have little reason to believe that the low frequency of the Gln allele in the nonsmoking control population is attributable to anything but imprecision from small numbers, we cannot eliminate the possibility that the *XPD* genotype affects smoking behavior. Furthermore, we should also note that our study was originally designed as a case-control study; therefore, our case-only design is a *post hoc* data analysis.

The logistic regression models in which smoking is treated as a continuous variable allowed us to further examine the *XPD*-smoking interaction. The comparison of an unconstrained model, in which both genotype-risk lines were free to have different slopes and intercepts, with a simplified model, in which there was no interaction term so that the genotype-risk lines were constrained to have the same slope but allowed to have different intercepts, showed that the unconstrained model fit the data significantly better, and thus supported an *XPD*-smoking interaction. To evaluate whether this interaction would still be observed in the absence of a gene effect among nonsmokers, we compared a single-intercept, two-slope model to a single-line model and again found support for an *XPD*-smoking interaction. These analyses further support that the interaction finding is not driven solely by the positive association between the Gln allele and bladder cancer among nonsmokers.

Our findings did not change when we incorporated into the analysis the information on *NAT1*-*NAT2* genotypes. In our gene-gene-exposure analyses we still found evidence for interactions between *NAT1*-*NAT2* and smoking, and *XPD* and smoking, but we found little evidence that these two genotypes modify the effect of each other. Therefore, it does not seem that having the *NAT1**10-*NAT2*-slow and *XPD* Lys/Lys or Lys/Gln genotypes increases bladder cancer risk in a synergistic manner. However, our findings are based on very small numbers for some of the strata, particularly for the *NAT1**10-*NAT2*-slow and *XPD* Gln/Gln combined genotypes, and, therefore, we do not have adequate power to test for three-way interactions. Furthermore, we carried out these analyses using black and white subjects combined and included ethnicity as a covariate in our models; therefore, we cannot rule out that our results could have been confounded by population admixture. Thus, our results should be interpreted with caution. Moreover, they are also based on the assumption that the dose response for exposure to smoking is linear in the log-odds scale for all genotypes. If the lack of interaction were true, it could either suggest that these proteins do not participate in the same pathways or, more likely, that there are backup or redundant mechanisms that compensate for the diminished or altered function of these different proteins.

There is only one other study in bladder cancer that has analyzed the *XPD* codon 751 polymorphism. In that study, carried out by Matullo *et al.* (33), a nonsignificant inverse association was found between the Gln allele and bladder cancer when comparing 124 cases to 47 nonurological controls. This finding is in agreement with our findings in the present study. When stratifying by smoking status, the authors report a positive association for the Gln allele among current smokers, but an inverse association among ex- or never-smokers. This is in contrast with our findings, and we cannot offer an explana-

tion for this discrepancy. One difference between the study by Matullo *et al.* and ours is that they grouped the genotypes differently (Lys/Lys versus Lys/Gln + Gln/Gln) than we did (Lys/Lys + Lys/Gln versus Gln/Gln). When we repeated our gene-smoking analyses comparing subjects with the Lys/Lys genotype to those with the Lys/Gln or Gln/Gln genotype, such as how Matullo *et al.* did, we no longer found evidence of a gene-smoking interaction. We observed an OR = 0.8 (95% CI = 0.4–1.9) within the nonsmoker stratum and an OR = 1.0 (95% CI = 0.6–1.6) within the smoker stratum, with a test of interaction $P = 0.71$. We observed similar results when classifying smoking according to three classes of pack-years smoked or years smoked. Therefore, in our study, we found evidence that the *XPD* codon 751 Gln allele seems to modify the effect of cigarette smoking, only when present in a homozygous genotype.

Three other epidemiological studies in different cancer types found evidence of inverse associations between the *XPD* codon 751 Gln allele and skin basal cell carcinomas (34), melanoma (35), and gliomas (36). One study found evidence of an inverse association between the codon 312 Asn allele, which was also found to be in linkage disequilibrium with the codon 751 Gln allele and lung cancer (37). Conversely, two epidemiological studies found evidence of a positive association between the Gln allele and head and neck squamous cell carcinomas (38) and lung cancer (20). Two other studies found weak positive associations between the Gln allele and basal cell carcinomas (39) and lung cancer (40).

The role of the codon 751 Gln allele remains unclear. The differences in risk observed among the different epidemiological studies could reflect exposure or tissue-specificity effects. Under this scenario, the presence of an allelic variant that induces subtle differences in protein activity may result in different cancer risks depending on the exposure or the metabolic pathways available in different tissues. Furthermore, the presence of variants in metabolism and other critical DNA repair genes may alter the influence of variants in the *XPD* gene. Biochemical studies that explore the consequences of the *XPD* codon 751 polymorphism will help resolve some of these issues. Another possibility is that the differences in risk observed in different studies could be partially attributable to the existence of another cancer susceptibility gene in linkage disequilibrium with *XPD*, which may vary in the different populations studied. Possible candidates are other polymorphisms in the *XPD* gene, such as the codon 312 polymorphism, which has been shown to affect the *XPD* protein (22). Other candidates are the *XRCC1* and the *ERCC1* genes, which map close to *XPD* on chromosome 19 (41). We have reported previously on *XRCC1* alleles in this same population (23), but found no evidence of linkage disequilibrium between *XRCC1* and *XPD* (data not shown). The effects of the *ERCC1* gene, which is also involved in NER and is less than 250 Kb from *XPD* (41), remain to be explored. The presence of another gene in linkage disequilibrium with *XPD* could also bias gene-smoking interaction analyses. Finally, the different associations between the *XPD* genotype and cancer, observed in various epidemiological studies including our own, could be attributable to chance or bias. Allele-specific studies of DNA-repair function will be an increasingly important adjunct to epidemiological studies in establishing whether common polymorphisms in *XPD* are plausible determinants of 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; Drs. Carla H. van Gils and David M. Umbach for helpful discussions; Sue Edelstein for graphic design; and Drs. Jane C. Schroeder and Gloria L. David-Beabes for critical review of this manuscript.

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