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Polymorphisms of DNA repair gene *XRCC1* in squamous cell carcinoma of the head and neck

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Because reduced DNA repair capacity (phenotype) has been suggested as a risk factor for squamous cell carcinoma of the head and neck (SCCHN), newly-identified DNA repair gene polymorphisms (genotype) may also be implicated in risk. To test this hypothesis, we conducted a case-control study of 203 SCCHN patients and 424 control subjects (matched for age, sex and ethnicity) to investigate the role of two *XRCC1* polymorphisms (*XRCC1* 26304 T and *XRCC1* 28152 A, respectively) in SCCHN. Multivariate logistic regression analysis was performed to calculate the adjusted odds ratio (OR) and 95% confidence interval (CI). A total of 180 cases (88.7%) and 363 controls (85.6%) lacked the *XRCC1* 26304 T allele [adjusted OR = 1.34 (CI, 0.80–2.25)]. Lack of this polymorphism was a significant risk factor specifically for cancers of the oral cavity and pharynx [adjusted OR = 2.46 (CI, 1.22–4.97)]. Thirty-two cases (15.8%) and 46 controls (10.8%) were homozygous for the *XRCC1* 28152 A allele [adjusted OR = 1.59 (CI, 0.97–2.61) for all cases, and 1.41 (CI, 0.80–2.48) for oral and pharyngeal cancer only]. Furthermore, when the two genotypes were combined into a three-level model of risk, a polymorphism–polymorphism interaction of increasing risk (trend test, $P = 0.049$) was evident: OR = 1.0 for those with neither risk genotype (referent group), adjusted OR = 1.51 (CI, 0.87–2.61) for those with either risk genotype, and 2.02 (CI, 1.00–4.05) for those with both risk genotypes. For oral and pharyngeal cancer, this trend was even more pronounced with the adjusted OR = 2.68 (CI, 1.28–5.61) for those with either risk genotype, and 3.22 (CI, 1.33–7.81) for those with both risk genotypes. The findings support the hypothesis that a polymorphic *XRCC1* DNA repair gene contributes to risk of developing SCCHN.

Introduction

While squamous cell carcinoma of the head and neck (SCCHN) accounts for only 4% of the incident cancers in the USA each year (1), it is the fifth most common cancer worldwide and second in the developing world (2). Consequently, SCCHN represents a major worldwide health problem, which will worsen if smoking rates in developing countries continue their upward trend (3). Studies have suggested that genetic susceptibility plays an important role in risk of SCCHN, because even though tobacco and alcohol exposures are the most important risk factors, only a small fraction of smokers will ever develop SCCHN (1,4). Further support for genetic susceptibility is evidenced by aggregation of SCCHN in first degree relatives of SCCHN patients (5) and by an early age at onset in a subgroup of patients (6).

Inherited polymorphisms in genes controlling both carcinogen metabolism and repair of DNA damage have been suggested to underlie this variability in susceptibility. Phenotypic abnormalities revealed by assays such as the mutagen sensitivity assay (7,8), host cell reactivation assay (9) and measurement of DNA repair gene transcript levels (10) have shown potential as markers of risk for SCCHN. Such assays imply that differences in DNA repair ability may partially underlie differences in risk; however, these phenotypic assays suffer from variability due to extrinsic influences. Conversely, genotype analysis of polymorphisms does not suffer from inter-test variability and is more applicable for screening purposes. While polymorphisms of several carcinogen metabolizing genes have been intensively studied (11,12), only recently have polymorphisms in several DNA repair genes in the normal population been reported (13,14).

Perhaps the best documented measure of susceptibility to SCCHN has been the mutagen sensitivity assay in which chromatid breaks are measured in cultured lymphocytes after exposure to the mutagens bleomycin (7) or benzo[*a*]pyrene diol epoxide (8). SCCHN patients are significantly more likely to exhibit the sensitive phenotype than are controls (7,8). Those patients with a family history of cancer (15), a young age at onset (16) or more than one tumor are especially likely to exhibit a sensitive phenotype (17). Simple chromatid breaks, as quantified in the mutagen sensitivity assay, result from double-strand breaks of DNA, and such damage is chiefly repaired via the recombinational repair pathway (18,19). Recently, polymorphisms of three genes (*ERCC1*, *XRCC3* and *XRCC1*) involved in this repair pathway were described (14). Shen *et al*. (14) found seven polymorphisms in *ERCC1*, none of which resulted in an amino acid change. They described four polymorphisms in *XRCC3*, only one of which resulted in an amino acid change, but whether this amino acid substitution occurs at a conserved site is unknown (14). However, they also found three polymorphisms of the *XRCC1* gene, which resulted in amino acid changes at evolutionary conserved regions (14). It is possible that these inherited polymorphisms of this pathway may affect risk of SCCHN. Therefore, we focused on the two reported polymorphisms with greater allele frequencies: a C→T substitution at position 26304 of the *XRCC1* gene (codon 194, exon 6) and a G→A substitution at position 28152 of the *XRCC1* gene (codon 399, exon 10).

The XRCC1 protein interacts with DNA ligase III in re-joining of DNA strand breaks (20) and DNA polymerase β in base excision repair (21). While the functional implication of polymorphisms in *XRCC1* are unknown, in theory amino

Abbreviations: CI, 95% confidence interval; OR, odds ratio; PCR, polymerase chain reaction; SCCHN, squamous cell carcinoma of the head and neck.

acid changes at conserved sites may alter its function. *XRCC1* mutants do display sensitivity to alkylating agents and ionizing radiation and exhibit elevated levels of sister chromatid exchange (22). Such altered function could be associated with increased cancer risk, and genotype frequency differences could theoretically be demonstrated between those with and without cancer. Thus, we performed restriction fragment length polymorphism analysis of two polymorphic sites of the *XRCC1* gene in a case-control study of SCCHN to test the hypothesis that genetic polymorphisms of this gene contribute to susceptibility to SCCHN.

Materials and methods

Study subjects

From May 1995 until October 1998, patients with histologically confirmed SCCHN (primaries of the oral cavity, oro/hypo-pharynx and larynx) were recruited from the registry of the Department of Head and Neck Surgery at the M.D.Anderson Cancer Center. Because this was a study of genotype and the marker was a constitutional one, both incident and prevalent cases were included. Cancer-free controls were selected from a pool of controls identified for ongoing hospital-based case-control studies by the Department of Epidemiology (23). These controls were selected from enrollees in a health maintenance organization and were matched to the cases by age, sex and ethnicity. Data on age, sex, ethnicity, smoking status and alcohol consumption were derived from questionnaires. Additionally, after informed consent was obtained, each subject donated 30 ml of blood in heparinized tubes for biomarker testing. For the cases, the medical record was reviewed to obtain clinical information on final histopathologic diagnosis, date of diagnosis and primary tumor site and stage. Patients with primaries of the nasopharynx or sinonasal tract, primaries outside the upper aerodigestive tract, cervical metastases of unknown primary origin and a histopathologic diagnosis other than squamous cell carcinoma were excluded. The research protocol was approved by the M.D.Anderson Institutional Internal Review Board.

Genotyping

The leukocyte cell pellet obtained from the buffy coat by centrifugation of 1 ml of whole blood, was used for DNA extraction. PCR assays were used to amplify exons of *XRCC1* containing the polymorphisms of interest. The primers for exons 5 and 6 were 5′-GCCAGGGCCCCTCCTTCAA-3′ and 5′-TACCCTCAGACCCACGAGT-3′, which generate a 485 bp fragment. The primers for exons 9 and 10 were 5′-CAGTGGTGCTAACCTAATC-3′ and 5′-AGTAGTCTGCTGGCTCTGG-3′, which generate an 871 bp fragment. These fragments (containing the polymorphic sites of interest) were amplified separately but under the same conditions as follows: a 50 μl reaction mixture containing ~50 ng of genomic DNA, 12.5 pmol of each primer, 0.1 mM each dNTP, 1× PCR buffer [50 mM KCl, 10 mM Tris–HCl (pH 9.0 at 25°C), 0.1 % Triton X-100], 1.5 mM $MgCl_2$, and 2.25 U *Taq* polymerase (Promega, Madison, WI). The mixtures were amplified with a Perkin Elmer GeneAmp PCR System 2400 (Perkin Elmer, Foster City, CA). The PCR profile consisted of an initial melting step of 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 61°C for 35 s and 72°C for 45 s, and a final elongation step of 72°C for 10 min. The PCR products were checked on a 1% agarose gel, photographed using Polaroid film and were then subjected to restriction fragment length polymorphism analysis.

The restriction enzyme *Pvu*II (New England BioLabs, Beverly, MA) was used to distinguish the 26304 polymorphism of exon 6 in which the gain of a *Pvu*II restriction site occurs in the polymorphic allele. The wild-type (C) allele (i.e. 26304 C) has a single band representing the entire 485 bp fragment including exons 5 and 6, and the polymorphic (T) allele (i.e. 26304 T) results in two fragments (396 and 89 bp, respectively). The restriction enzyme *Nci*I (New England BioLabs) was used to distinguish the 28152 polymorphism of exon 10 in which the loss of a *Nci*I restriction site occurs in the polymorphic allele. The wild-type (G) allele (i.e. 28152 G), which has two *Nci*I restriction enzyme sites, has three bands (461, 278 and 132 bp) and the polymorphic (G) allele (i.e. 28152 A) has only two bands (593 and 278 bp, respectively). Digestion of the PCR product was carried out using 5 U *Pvu*II or 10 U *Nci*I and the 1× buffer supplied with each restriction enzyme at 37°C overnight. The digestion products were separated on a 3% NuSieve 3:1 agarose (FMC BioProducts, Rockland, ME) gel and photographed using Polaroid film.

Statistical analysis

The genotype was analyzed as a dichotomized variable of either 0 for homozygous (26304 CC or 28152 AA) or 1 for the remaining genotypes. Univariate analysis was first performed to calculate the crude odds ratios (ORs) and their 95% confidence intervals (CIs) for the various genotypes or their combinations. Those subjects who had smoked more than 100 cigarettes in their lifetimes were defined as 'ever' smokers; of these, those who had quit smoking for >1 year prior to diagnosis were defined as former smokers and the others as current smokers including recent quitters (within 1 year). Those who drank alcoholic beverages at least once a week for >1 year prior to diagnosis were defined as 'ever' users of alcohol; of these, those who had quit drinking for >1 year were defined as former drinkers and the others as current drinkers including recent quitters (within 1 year). Ethnicity was recorded as non-Hispanic white, African-American or Hispanic. For logistic regression analysis, the *XRCC1* genotype was recoded as a dummy variable. To evaluate the combined effect of these polymorphisms, the subjects were categorized into three mutually exclusive risk genotypes: 3 (1,0) for both 26304 CC and 28152 AA, 2 (0,1) for either risk genotype and 1 (0,0) for neither risk genotype. To assess trend, this trichotomous variable was treated as a continuous variable of combined risk genotypes (1, neither; 2, either; 3, both), assuming there was a 'gene dosage', and fit in the logistic regression model. All of the statistical analyses were performed with Statistical Analysis System software (v.6; SAS Institute, Cary, NC).

Results

During the study period, 203 patients with oral, pharyngeal or laryngeal cancer met the eligibility criteria for this study, and 424 controls were selected to match these cases. Frequency distribution analysis was carried out and summarized in Table I. The mean age in years was 59.8 (median 61, range 20–89) for the cases and 60.1 (median 62, range 27–84) for the controls. There were no significant differences between cases and controls by sex or ethnicity. Although an effort was made to achieve a frequency match on smoking status between cases and controls, smoking and alcohol use were more prevalent among the cases than the controls. Therefore, these variables were further adjusted in later multivariate analyses.

Only one case but no controls were homozygous for the 26304 polymorphism (26304 TT) of *XRCC1*, while 22 cases (10.8%) and 61 controls (14.4%) were heterozygous for this polymorphism (26304 CT) ($P = 0.280$, χ^2 test; Table II). The slightly higher frequency of this polymorphism in the controls suggested that it may not be an adverse genotype, whereas the

Table I. Frequency distribution analysis of select demographic and risk factors in SCCHN patients and healthy controls

Variable	Cases		Controls		*P*-value[a]
	No.	(%)	No.	(%)	
Age (years)					0.387
⩽45	20	(9.9)	46	(10.8)	
46–55	54	(26.6)	91	(21.5)	
56–65	63	(33.0)	125	(29.5)	
>65	66	(32.5)	162	(38.2)	
Sex					0.321
Male	137	(67.5)	269	(63.4)	
Female	66	(32.5)	155	(36.6)	
Ethnicity					0.954
Non-Hispanic white	184	(90.6)	381	(89.8)	
Mexican-American	12	(5.9)	27	(6.4)	
African-American	7	(3.5)	16	(3.8)	
Smoking status					0.011
Current[b]	81	(39.9)	120	(28.3)	
Former	73	(36.0)	193	(45.5)	
Never	49	(24.1)	111	(26.2)	
Alcohol status					0.001
Current[b]	114	(56.2)	173	(40.8)	
Former	47	(23.1)	123	(29.0)	
Never	42	(20.7)	128	(30.2)	

[a]Two-sided χ^2 test.
[b]Includes recent quitters.

homozygous wild-type (26304 CC), more frequent in the cases (88.7%) than in the controls (85.6%), was the assumed risk genotype. After dichotomizing the data by the *XRCC1* 26304 CC genotype versus the other genotypes, the crude OR for the 26304 CC genotype associated with SCCHN was 1.32 (CI, 0.79–2.19) (Table III). In a logistic regression model that included age, sex, ethnicity, smoking status and alcohol status, the 26304 CC genotype was not a statistically significant risk factor for SCCHN [adjusted OR = 1.34 (CI, 0.80–2.25)]. However, patients with primaries of the oral cavity and pharynx (n = 150) exhibited a higher frequency (93.3%) of the 26304 CC genotype than did laryngeal cancer patients (75.5%; n = 53), and having the 26304 CC genotype was a significant risk for cancer of the oral cavity or pharynx [crude OR = 2.35 (CI, 1.17–4.72)]. Furthermore, this genotype remained an independent marker of risk for oral and pharyngeal cancer in multivariate logistic regression analysis [adjusted OR = 2.46 (CI, 1.22–4.97)] (Table III), whereas no significantly elevated risk was associated with this genotype in laryngeal cancer (data not shown).

Thirty-two cases (15.8%) and 46 controls (10.8%) were homozygous for the 28152 polymorphism (28152 AA) of *XRCC1* (P = 0.083, χ^2 test; Table II), suggesting that being homozygous for this polymorphism may be a risk genotype. After dichotomizing the data by the *XRCC1* 28152 AA genotype versus the other genotypes, the crude OR for the 28152 AA genotype associated with SCCHN was 1.54 (CI, 0.95–2.50) (Table III). This risk was virtually unchanged after adjustment for age, sex, ethnicity, smoking status and alcohol status in the multivariate logistic regression analysis [adjusted OR = 1.59 (CI, 0.97–2.61)]. Likewise, the 28152 AA genotype conferred a similarly elevated, but not statistically significant, risk (OR = 1.41; CI, 0.80–2.48) for oral and pharyngeal cancer only (Table III).

The data on these two polymorphisms were combined into four mutually exclusive genotype groups: 81 subjects had neither *XRCC1* 26304 CC nor *XRCC1* 28152 AA; 468 subjects had 26304 CC; three subjects had the 28152 AA; and 75 subjects had both 26304 CC and 28152 AA. Because only three subjects were 28152 AA homozygous, they were grouped with 26304 CC homozygous as 'either 28152 AA or 26304 CC' (n = 471). Although these two genotypes were not independent, only 75 subjects had both 26304 CC and 28152 AA. These individuals who had two risk genotypes should have an excess risk. Indeed, as shown in Table III, the risk for the disease increased as the number of risk genotypes increased (trend test, P = 0.062). After adjustment for age, sex, ethnicity, smoking status and alcohol status in the multivariate logistic regression analysis, using the absence of either risk genotype as the referent group: having either risk genotype increased an individual's risk by 1.5-fold, whereas having both risk genotypes increased an individual's risk by 2-fold (trend test, P = 0.049). For oral and pharyngeal cancer, this trend was even more pronounced (trend test, P = 0.012): the adjusted ORs were 2.68 (CI, 1.28–5.61) for those with either risk genotype and 3.22 (CI, 1.33–7.81) for those with both risk genotypes (Table III).

After stratifying for the potential confounding variables presented in Table I, some interesting differences in genotype frequencies were noted between subgroups of patients (Table IV). In particular, younger patients (age ⩽50 years) tended to have higher frequencies of both the 26304 CC (93.8%) and 28152 AA (22.9%) risk genotypes compared with 82.4 and 11.0% for the controls, respectively. These high frequencies of *XRCC1* risk genotypes were associated with elevated but non-significant risks for the disease. While females appeared to have higher frequencies of the 26304 CC genotype and males

Table II. *XRCC1* genotype and allele frequencies factors in SCCHN patients and healthy controls

Genotype	Cases		Controls	
	No.	(%)	No.	(%)
XRCC1 26304				
CC	180	(88.7)	363	(85.6)
CT	22	(10.8)	61	(14.4)
TT	1	(0.5)	0	(0.0)
T allele frequency		5.9%		7.2%
XRCC1 28152				
GG	94	(46.3)	181	(42.7)
GA	77	(37.9)	197	(46.5)
AA	32	(15.8)	46	(10.8)
A allele frequency		34.7%		34.1%

Table III. Logistic regression analysis of XRCC1 polymorphisms in SCCHN

Genotype	All cases				Oral and pharyngeal cancer cases				Controls	
	No.	(%)	Crude OR (CI)	Adjusted OR (CI)[a]	No.	(%)	Crude OR (CI)	Adjusted OR (CI)[a]	No.	(%)
XRCC1 26304										
TT or CT	23	(11.3)	1.00	1.00	10	(6.7)	1.00	1.00	61	(14.4)
CC	180	(88.7)	1.32 (0.79–2.19)	1.34 (0.80–2.25)	140	(93.3)	2.35 (1.17–4.72)	2.46 (1.22–4.97)	363	(85.6)
XRCC1 28152										
GG or GA	171	(84.2)	1.00	1.00	129	(86.0)	1.00	1.00	378	(89.2)
AA	32	(15.8)	1.54 (0.95–2.50)	1.59 (0.97–2.61)	21	(14.0)	1.34 (0.77–2.33)	1.41 (0.80–2.48)	46	(10.8)
Genotype combinations										
Neither 26304 CC nor 28152 AA	20	(9.8)	1.00	1.00	9	(6.0)	1.00	1.00	61	(14.4)
Either 26304 CC or 28152 AA	154	(75.9)	1.48 (0.86–2.54)	1.51 (0.87–2.61)	121	(80.7)	2.59 (1.25–5.37)	2.68 (1.28–5.61)	317	(74.8)
Both 26304 CC and 28152 AA	29	(14.3)	1.92 (0.97–3.82)	2.02 (1.00–4.05)	20	(13.3)	2.95 (1.23–7.07)	3.22 (1.33–7.81)	46	(10.8)
Trend test[b]			P = 0.062	P = 0.049			P = 0.019	P = 0.012		

[a]Adjusted for age, sex, ethnicity, smoking and alcohol use.
[b]Using a categorical variable of combined risk genotypes (1, neither; 2, either; 3, both).

Table IV. Stratification analysis of XRCC1 genotype frequencies, OR and CI in SCCHN

Variable	XRCC1 26304 CC genotype							XRCC1 28152 AA genotype						
	Case (n = 203)			Control (n = 424)			Adjusted OR (CI)[a]	Case (n = 203)			Control (n = 424)			Adjusted OR (CI)[a]
	No.	Others	%	No.	Others	%		No.	Others	%	No.	Others	%	
Age (in years)														
⩽50	45	3	93.8	75	16	82.4	3.63 (0.97–13.7)	11	37	22.9	10	81	11.0	2.45 (0.91–6.58)
51–65	76	13	85.4	145	26	84.8	1.01 (0.48–2.14)	12	77	13.5	16	155	9.4	1.70 (0.73–3.95)
>65	59	7	89.4	143	19	88.3	1.18 (0.46–3.05)	9	57	13.6	20	142	12.4	1.07 (0.45–2.52)
Sex														
Male	120	17	87.6	228	41	84.8	1.19 (0.64–2.22)	24	113	17.5	31	238	11.5	1.55 (0.85–2.82)
Female	60	6	90.9	135	20	87.1	1.54 (0.58–4.11)	8	58	12.1	15	140	9.7	1.33 (0.52–3.38)
Ethnicity														
White	164	20	89.1	330	51	86.6	1.29 (0.74–2.25)	31	153	16.9	44	337	11.6	1.61 (0.97–2.67)
Other[b]	16	3	84.2	33	10	76.7	1.98 (0.38–10.3)	1	18	5.3	2	41	4.7	1.73 (0.13–22.5)
Smoking status														
Current[c]	74	7	91.4	102	18	85.0	1.72 (0.80–3.67)	15	66	18.5	9	111	7.5	3.18 (1.28–7.94)
Former	61	12	83.6	165	28	85.5	1.12 (0.44–2.88)	8	65	11.0	20	173	10.4	1.03 (0.43–2.54)
Never	45	4	91.8	96	15	86.5	0.82 (0.26–2.54)	9	40	18.4	17	94	15.3	1.18 (0.49–2.91)
Alcohol status														
Current[c]	103	11	90.4	146	27	84.4	1.73 (0.82–3.65)	20	94	17.5	17	156	9.8	2.21 (1.07–4.56)
Former	40	7	85.1	102	21	82.9	1.18 (0.46–2.99)	6	41	12.8	10	113	8.1	1.81 (0.61–5.43)
Never	37	5	88.1	115	13	89.8	0.84 (0.28–2.50)	6	36	14.3	19	109	14.8	0.80 (0.29–2.18)

[a]Adjusted for other covariates presented in this table in a logistical regression model for each stratum.
[b]Mexican-Americans and African-Americans were combined for this analysis due to their relatively small sample size.
[c]Includes recent quitters.

appeared to have higher frequencies of the 28152 AA genotype, these differences were not statistically significantly different. In both case and control categories, African-Americans and Mexican-Americans had lower frequencies of the risk genotypes than whites, but the numbers of subjects in these ethnic groups were relatively small (Table IV). Current smokers and drinkers tended to have the highest frequency of the risk genotypes among cases, while never users always had the highest frequency among controls. Consequently, the greatest difference between cases and controls was always found in current users of tobacco and alcohol (Table IV). The multivariate analyses indicated that there was a significantly increased risk associated with the *XRCC1* 28152 AA genotype among current users of tobacco and alcohol [adjusted ORs 3.18 (95% CI, 1.28–7.94) and 2.21 (95% CI, 1.07–4.56), respectively] but not in other subgroups. A total of 150 patients were incident cases, and 53 patients were prevalent cases. Whereas prevalent cases had slightly lower frequencies of the risk genotypes, these differences were not statistically significant (data not shown). Among patients with a newly diagnosed SCCHN (n = 150), there was no statistically significant difference in genotype frequencies related to T stage, N stage or overall stage (data not shown).

Discussion

This study demonstrated an elevated risk of SCCHN associated with the frequencies of two *XRCC1* polymorphisms, particularly in subgroups of current users of tobacco and alcohol. Furthermore, when these two risk genotypes were combined, there was an additive effect on the risk of SCCHN. To the best of our knowledge, this is the first large molecular epidemiological study of SCCHN on these newly identified polymorphisms in *XRCC1*. These findings suggest that polymorphisms in *XRCC1* play a role in the etiology of smoking-related SCCHN.

The findings are biologically plausible. If amino acid differences (especially at conserved sites) in these enzymes impair their function in the repair of DNA damage, these polymorphisms should theoretically predispose an individual to a tobacco-induced cancer. While studies of actual protein function and related DNA repair phenotype are needed to confirm that these amino acid differences do indeed result in changes in repair proficiency, several lines of evidence presented herein support this biologically plausible concept. First are the higher frequencies of the risk genotypes (26304 CC, 28152 AA and their combination) in the cases than in the controls, a finding consistent across most subgroups. Furthermore, the dose–response-like effect of risk associated with the risk genotypes supports an additive effect of these different polymorphisms. Second, some of the highest risk genotype frequencies among cases were in the relatively young, a theoretically more susceptible group. Third, the difference in the frequencies between cases and controls was greatest for those who were current smokers or drinkers, consistent with a marker of genetic susceptibility reflecting a gene–environment interaction. In other words, having the risk genotype is not a risk factor for cancer unless one also has exposure. Finally, Lunn recently reported that *XRCC1* 28152 (codon 399) polymorphism was associated with higher levels of both aflatoxin B1–DNA adducts and glycophorin A variants in a normal population (24).

However, our results may be biased by the relatively small number of subjects in the various subgroups and therefore need to be duplicated by others. Further studies with a larger sample (particularly of young patients, non-smoking and non-drinking patients and minorities) and more complete measures of tobacco exposure are needed to clarify these complex interactions between genotype, exposure and demographic characteristics.

If *XRCC1* genotype is indeed a marker of genetic susceptibility rather than a tumor marker, its frequency should not

segregate with disease status (incident/prevalent) or stage of disease, and it did not. However, certain genotypes could confer a greater susceptibility to disease at particular sites. In this study, the risk estimates tended to be higher for oral and pharyngeal cancer, and this difference may reflect the etiologic differences (25,26) between individual head and neck cancer sites. Clearly, this finding is preliminary and larger studies will be needed to explore these differences in genotype frequency for patients with tumors of various sites (particularly larynx).

As for the control population, this study included 424 cancer-free subjects (381 Caucasian subjects, 27 Mexican-Americans and 16 African-Americans) and, thus, we can provide an estimate of the *XRCC1* polymorphism frequencies particularly for Caucasians in the general population. Shen *et al*. (14) reported an allele frequency of 25% for both *XRCC1* 26304 T and *XRCC1* 28152 A in a small study of 12 apparently normal individuals. Lunn *et al*. (24) reported an allele frequency of 6% for *XRCC1* 26304 T and 37% for *XRCC1* 28152 A for Caucasians (n = 169). In this study, we found an allele frequency of 7.2% for *XRCC1* 26304 T and of 34.1% for *XRCC1* 28152 C in 380 Caucasians, which are consistent with the report by Lunn *et al*. (24).

In conclusion, this study represents an important addition to previously published work on DNA repair phenotypes as markers of genetic susceptibility (7–10) and is the first large case-control study to evaluate the cancer risk of individuals having *XRCC1* genotypes. Such genotyping analysis of DNA repair genes and correlation with repair phenotype in future molecular epidemiologic studies will enhance our ability to identify those individuals most susceptible to tobacco-induced carcinogenesis.

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