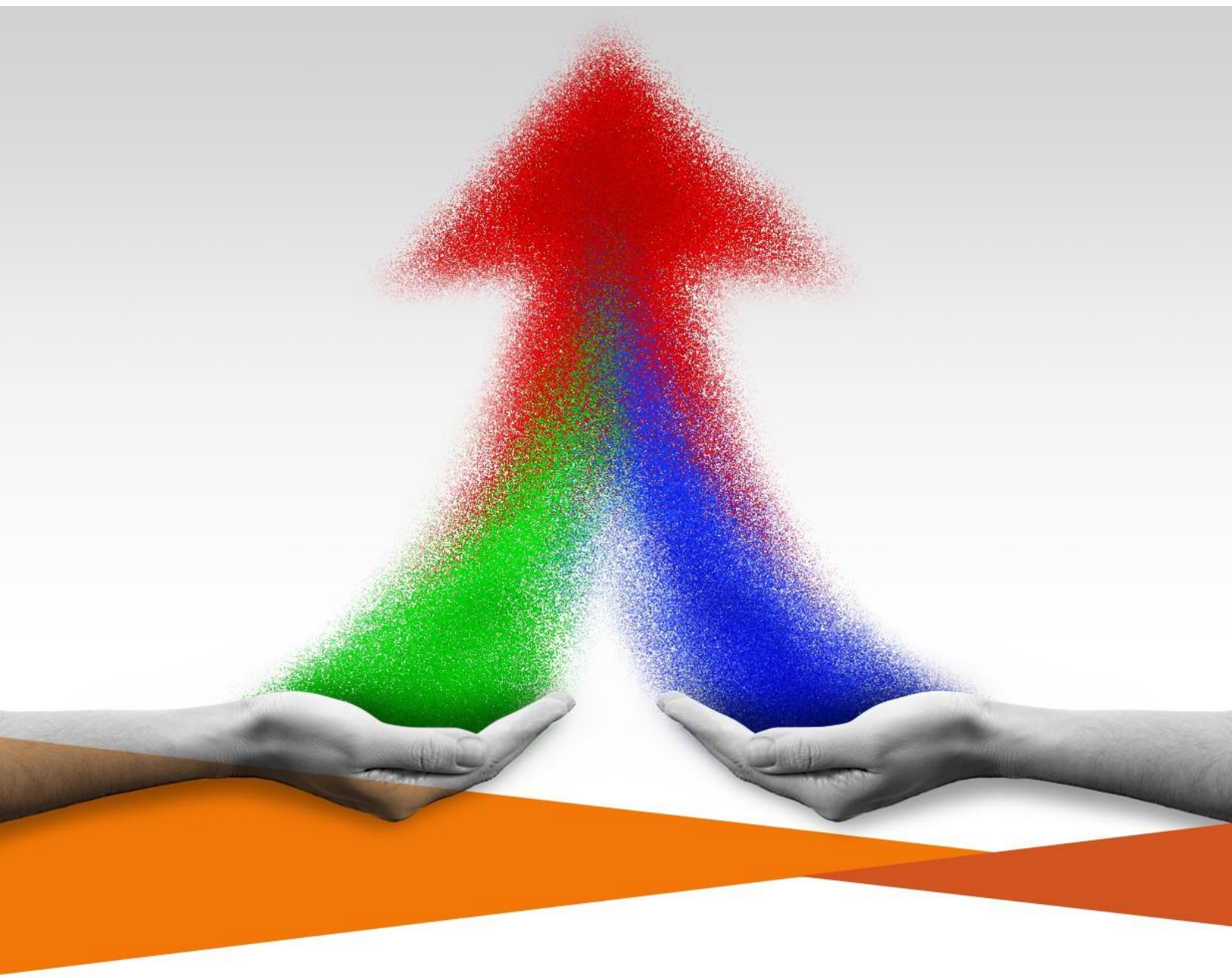

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Smoking modifies the relationship between *XRCC1* haplotypes and HPV16-negative head and neck squamous cell carcinoma

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Reports on the relationship between head and neck squamous cell carcinoma (HNSCC) and polymorphisms in *X-ray cross complementing group 1* (*XRCC1*) have been inconsistent. We hypothesized this may be due to not accounting for Human papillomavirus type-16 (HPV16) and thus examined whether smoking modified the association between *XRCC1* haplotypes and HNSCC risk within HPV16 serologic strata. Cases were diagnosed in Greater Boston, Massachusetts. Controls were matched to cases on age, gender and residential town. Genotyping was conducted on three *XRCC1* polymorphisms (Arg194Trp, Arg280His and Arg399Gln) and serology was used to determine HPV16 exposure. Unconditional logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs), adjusting for age, sex, race, education, smoking, alcohol consumption and HPV16 serology. There was no overall association between *XRCC1* polymorphisms and HNSCC risk. Smoking did not modify the association between *XRCC1* polymorphisms and HNSCC risk among the HPV16 seropositive ($p_{interaction} = 0.89$) but it did for the HPV16 seronegative ($p_{interaction} = 0.04$). Among the HPV16 seronegative, heavy smokers with a haplotype containing a variant allele had an increased HNSCC risk (haplotype with 399Gln: OR, 1.35; 95% CI, 0.97–1.86), whereas never/light smokers with variant alleles may have a reduced risk. In sum, the association between *XRCC1* and HNSCC risk differed by HPV16 status and smoking. Among the HPV16 seronegative, heavy smokers with *XRCC1* variant alleles had an increased HNSCC risk. There was no relationship between *XRCC1* and HPV16-related HNSCC, regardless of smoking. Our findings underscore the importance of accounting for HPV16 exposure even when studying susceptibility to HNSCC.

Key words: Human papillomavirus (HPV); head and neck squamous cell carcinoma (HNSCC); tobacco; *XRCC1*; DNA repair

Approximately 434,000 new cases of head and neck squamous cell carcinoma (HNSCC) are expected worldwide annually[1] and over 45,000 of these will occur in the U.S.[2] Tobacco and alcohol together are thought to explain 75% of HNSCC incidence.[3] The predominant risk factor, tobacco, contains numerous known carcinogens including benzene, polycyclic aromatic hydrocarbons and nitrosamines. A review of the literature found that smokers are at a 3- to 12-fold greater risk of HNSCC than nonsmokers and among those who do not drink alcohol, the relative risks for smoking were between 2 and 5.[4]

Because of the strong association with tobacco, it has been hypothesized that genetic variation in the DNA repair gene *X-ray cross complementing group 1* (*XRCC1*) translates into greater HNSCC susceptibility. XRCC1 is involved in base excision repair (BER), which repairs base damage, strand breaks and nonbulky DNA adducts induced by a number of agents including tobacco. XRCC1 acts as a scaffold and coordinates protein interactions in the BER pathway, including DNA ligase III, DNA polymerase β and poly ADP-ribose polymerase (PARP).[5,6]

Three single nucleotide polymorphisms (SNPs) in *XRCC1* have been studied more widely because they are common (minor allele frequency > 0.05) and nonsynonymous, resulting in amino acid changes that may alter the protein's ability to perform its functions. These polymorphisms, Arg194Trp, Arg280His and Arg399Gln, are all located in evolutionarily conserved regions. Research suggests that polymorphisms in *XRCC1* may influence DNA repair. For instance, the 399Gln variant was associated with greater levels of glycophorin A variants and DNA adducts,[7–9] as well as sister chromatid exchanges.[9–11] Others did not observe a relationship between DNA adducts and either the 399Gln or 194Trp polymorphisms.[12] In a study of oral cancers, researchers found that the 399Gln allele was associated with a higher frequency of *TP53* mutations,[13,14] providing additional support that *XRCC1* polymorphisms may alter risk of HNSCC.

Epidemiologic findings for the association of these *XRCC1* polymorphisms with HNSCC have been inconsistent. For example, some researchers have reported that HNSCC risk was increased among those with 194Trp[15–17] and 280His,[17] whereas others have reported no association with these polymorphisms.[15,18,19] The most research has been conducted on the 399Gln polymorphism, in part because of its location in a BRCT binding domain thought to be important in protein interaction.[6,20] Olshan *et al.*[18] found that the 399Gln polymorphism was associated with a reduced risk of HNSCC for both blacks and whites. This was later supported by a pooled analysis of 2 studies in the U.S. and one in Puerto Rico where the homozygous-variant genotype was reported to be protective and statistically significant.[21] Contradicting this relationship were studies that found the 399Gln allele to increase HNSCC risk[17,22] or have no relationship with HNSCC risk,[15,16,19,23] including a meta-analysis of 7 studies which reported no association for both studies of whites and Asian populations.[23] Explanations for these conflicting findings included that the studies had low power, the populations studied differed in their tobacco use or exposure to other DNA damaging agents or the polymorphisms were in linkage disequilibrium with another polymorphism.[21]

However, another possible explanation is that previous studies did not examine this relationship while accounting for the presence of exposure to Human papillomavirus-type 16 (HPV16). Research suggests that HPV16-related HNSCC differs clinically and etiologically. For instance, survival among HNSCC patients has been found to be better among those with HPV16-related disease.[24–31] In addition, the influence of the predominant risk

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factors, alcohol and tobacco, have been found to be diminished or null among HPV16-related HNSCC.[32] Another example involves the relationship between HPV16, HNSCC risk and Vitamin C consumption. When HPV16 exposure was ignored, the association between Vitamin C and HNSCC appeared null[33]; however, when stratified by HPV16, Vitamin C was associated with a decreased HNSCC risk among the HPV16 seronegative but an increased HNSCC risk among the HPV16 seropositive.[34] Mounting evidence strongly suggests that HPV16-related HNSCC is a distinct disease. Therefore, if the HPV16-positive individuals are included in an analysis assessing an interaction between *XRCC1* polymorphisms and smoking, bias toward the null may occur.

Our objective was to determine whether the *XRCC1* polymorphisms Arg194Trp, Arg280His and Arg399Gln, analyzed as haplotypes, influence the risk of HNSCC. In particular, we evaluated whether smoking modifies this relationship, and if this differed for HPV16-related and HPV16-unrelated HNSCC.

Material and methods

Study population

Incident cases of HNSCC in Greater Boston, Massachusetts diagnosed between December 1999 and December 2003 were identified from head and neck clinics and departments of otolaryngology or radiation oncology at 9 Boston-area academic medical facilities (New England Medical Center, Massachusetts General Hospital, Massachusetts Eye and Ear Infirmary, Dana-Farber Cancer Institute, Brigham and Women's Hospital, Boston Veterans Administration, Beth Israel Deaconess Medical Center, Boston Medical Center and Harvard Vanguard Medical Associates).

Eligibility criteria for cases included carcinoma of the tongue, gum, floor of mouth, other location in the mouth, oropharynx, hypopharynx, ill-defined site within lip oral cavity and pharynx and larynx (corresponding to *International Classification of Disease, Ninth Revision* (ICD-9) codes 141, 143, 144, 145, 146, 148, 149 and 161, respectively), as determined by review of pathology reports. The diagnosis needed to occur not more than 6 months prior to when the patient was contacted for study participation. Patients with recurrent disease were excluded. Additional criteria included being at least 18 years of age and a resident of the study area, which included 249 cities and towns within a 1 hr drive of Boston.

Population-based controls were identified through Massachusetts town books, which are required by state law to list all residents 17 years and older. Potential controls were contacted if they came from the same town as the matched case and were randomly sampled from those who also matched the case's sex and age (± 3 years). Study protocol and materials were approved by the institutional review board at the 9 medical facilities and the Harvard School of Public Health, and all study participants provided written informed consent.

A total of 823 HNSCC cases were found to be eligible for the study, although 57 refused to participate and 44 did not complete their questionnaire. The remaining 722 enrolled in the study. For population-based controls, 1,643 eligible subjects were contacted and 815 consented to participate. Six controls were excluded when their corresponding case later became ineligible. Among the remaining subjects, 765 completed the questionnaire. From 2001 on, blood samples were requested, and these were obtained from 81% of cases and 80% of controls enrolled during that time. Blood samples allowed for the detection of HPV16 antibodies, as well as the genotyping and estimation of *XRCC1* haplotypes for 485 cases and 549 controls.

Questionnaire

Subjects answered a self-administered questionnaire that inquired about demographic characteristics, medical history, diet, and smoking and drinking habits. Cases received their questionnaires during a clinic visit. Controls were mailed their questionnaires and a research coordinator reviewed their responses in-person on a subsequent visit.

A detailed description of the data collection for smoking and drinking has been discussed previously.[32] A brief description is provided here. The questionnaire sections for smoking and drinking were decade-specific. Subjects reported their average consumption of beer, wine and liquor in a typical week for each decade of their life. These data were used to calculate the subject's lifetime average drinks per week. If subjects refused to answer the decade-specific alcohol section (50 cases but no controls refused), they reported their usual alcohol consumption: number of days of the week they would drink and the number of drinks they would typically consume. For smoking, subjects who said they had not smoked more than 100 cigarettes (5 packs) in their lifetime were considered never smokers. The remaining subjects were asked to report the number of packs smoked per day during each decade of life, and from this information, we calculated pack-years (pack/day-years). If a subject refused to complete the lifetime smoking section (43 cases and 1 control refused), they reported their average cigarettes smoked per day when they were a regular smoker and how many years they smoked.

HPV16 serology

The method used to ascertain the HPV16 serological status of cases and controls has been described previously.[35] Serum was separated within 24 hr of collection and frozen at -80°C. The HPV Competitive Luminex® Immunoassay was used to determine presence of antibodies to the L1 protein of HPV16.[36] Positive and negative controls were used for quality controls and testing of samples was done in duplicate.

XRCC1 *genotyping*

Genomic DNA was extracted from whole blood using the QIAamp Blood Kit (QIAGEN, Valencia, California). Arg194Trp (rs1799782, C to T, exon 6), Arg280His (rs25489, G to A, exon 9) and Arg399Gln (rs25487, G to A, exon 10) were genotyped using PCR-RFLP for each SNP individually. The forward (F) and reverse (R) primers were as follows: Arg194Trp F: 5′-TGAAG GAGGAGGATGAGAGC and R: 5′-CTCTACCCTCAGACC CACGA; Arg280His F: 5′-CCCCAGTGGTGCTAACCTAA and R: 5′-ACACCCTGAAGGATCTTCCC; and Arg399Gln F: 5′-CCAAGTACAGCCAGGTCCTA and R: 5′-AGTCTGACTCCC CTCCGGAT. The PCR conditions started with incubation at 94°C for 5 min, followed by 35 cycles at 94°C for 30 sec, 60°C for 30 sec and 72°C for 45 sec, and completing with a hold at 72°C for 1 min. PCR products were digested with *RSA I* for Arg280His and *Msp I* for both Arg194Trp and Arg399Gln (New England Biolabs, Beverly, MA). Each digested sample was separated by electrophoresis in an agarose gel containing Tri-borate EDTA buffer and ethidium bromide. For quality control, laboratory personnel were blinded to case-control status and negative and positive controls were used to ensure replication.

Statistical analysis

We tested whether the SNPs were in Hardy-Weinberg equilibrium among the controls. Unconditional logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) to examine the relationship between *XRCC1* SNPs and HNSCC risk. Unconditional logistic regression is appropriate for frequency matching when matching variables are included in the model.[37] Models controlled for age (continuous), sex, race (white or other), education (dichotomized by whether earned high school diploma), HPV16 serologic status (negative or positive), alcohol consumption (<3, 3 to <8, 8 to <25, ≥ 25 average drinks per week) and tobacco use (never smoker, >0 to <20, 20 to <45 and ≥ 45 pack-years). Since attempts to determine cutpoints for these variables in controls resulted in half of cases assigned to the highest category, the categories of alcohol consumption and tobacco use were based on the distribution in cases and controls combined.

More finely adjusting for smoking and drinking did not change the observed associations. We adjusted for differences in education using a dichotomized variable indicating whether or not a subject had earned a high school diploma. Controlling for household income or adjusting for more categories of education did not change the results. Restricting to whites only did not change the findings presented here, therefore, we included subjects regardless of reported race. All statistical tests were two-sided.

To compare our results with other studies, we first generated models containing indicator variables for the heterozygous genotype and homozygous variant genotype compared with the homozygous wild-types and we did this for each of the polymorphisms individually. Haplotypes of *XRCC1* were estimated using the HAPPY macro (http://www.hsph.harvard.edu/faculty/kraft/soft.htm[38]) in SAS version 9.1. Haplotype trend regression[39,40] was used to calculate ORs and 95% CIs for common haplotypes (>0.05%), assuming a codominant model. A global Chi-square test was used to examine whether the *XRCC1* haplotypes modified risk of HNSCC. Next, we investigated whether HPV16 serologic status influenced an association between *XRCC1* haplotypes and HNSCC. We created interaction terms by taking the cross-product for each haplotype and HPV16 serologic status and including these terms in the model along with terms for the individual haplotypes and HPV16 serology. We conducted a test for interaction by comparing the −2 log likelihoods for models with the cross-products compared with those without (a 3-degree of freedom test).

We next investigated whether smoking modified an association between *XRCC1* haplotypes and HNSCC risk. To increase power, we dichotomized smoking (<20 or ≥20 pack-years). Because previous research suggested that the association between smoking and HNSCC risk differed by HPV16 status,[32] we investigated this interaction, stratified by HPV16 serology. However, we lacked the power to look at this relationship in the HPV16 seropositive. Therefore, the *XRCC1* data were simplified into a single binary indicator for having at least one copy of a variant allele for any of the polymorphisms. To test for interaction, a model containing the dichotomized variable for smoking, the indicator variable for having an *XRCC1* variant and their cross-product was compared with a model that did not include the cross-product (a 1-degree of freedom test).

We have previously reported that the associations between HNSCC with tobacco and HPV16 serology vary by tumor site, with tobacco use being more strongly associated with laryngeal tumors and HPV16 serology as the greatest risk factor for pharyngeal tumors.[32,35] Therefore, in this analysis, we also examined the relationship between HPV16 serology, *XRCC1* polymorphisms, smoking and HNSCC risk by tumor site. As we have described,[32] tumors were classified into laryngeal, oral cavity and pharyngeal. Following the recommendations of the American Joint Committee on Cancer, tumors at the base of the tongue were considered pharyngeal and those located at the anterior of the tongue were classified as of the oral cavity. Two cases with carcinoma of the tongue were excluded from the site analyses because the pathology review did not allow for further classification. We examined the association between site-specific HNSCC risk and having an *XRCC1* variant allele, stratified by pack-years status (<20 or ≥20 pack-years), among the HPV16 seronegative. Polytomous logistic regression was used to estimate ORs and 95% CIs, controlling for age, sex, race, education and alcohol consumption. To assess statistical interaction between pack-years and having an *XRCC1* SNP, we examined the Wald test statistic for the interaction term between pack-years and *XRCC1*.

Results

The distribution of characteristics in the study population is presented in Table I. The mean age of cases was about 60 years of age and they were predominantly male (74%). The proportion of whites compared with nonwhites did not differ statistically between cases and controls after controlling for age and sex ($p = 0.85$); however, cases were less likely than controls to have earned a high school diploma ($p < 0.01$). In addition, cases had accumulated more pack-years and consumed more alcoholic beverages per week, on average, than controls ($p < 0.01$). Serologic results suggested that a higher proportion of cases than controls had a positive antibody titer to HPV16 ($p < 0.01$).

TABLE I – SELECTED DESCRIPTIVE CHARACTERISTICS FOR A STUDY OF HEAD AND NECK SQUAMOUS CELL CARCINOMA IN THE GREATER BOSTON AREA

	Cases		Controls		p-value[1]
	$n = 485$	%	$n = 549$	%	
Age					
Mean (SD)	59.5	(± 11.6)	61.0	(± 11.5)	
Gender					
Male	360	74.2	402	73.2	
Female	125	25.8	147	26.8	
Race					
White	444	91.6	501	91.3	0.85
Other	41	8.5	48	8.7	
High school diploma[2]					
Yes	361	82.0	503	91.8	<0.01
No	79	18.0	45	8.2	
Cigarettes, pack-years					
Never	90	18.5	182	33.2	<0.01
>0 to <20	92	19.0	152	27.7	
20 to <45	126	26.0	123	22.4	
≥45	177	36.5	92	16.8	
Alcohol, average drinks/week					
<3	90	18.6	154	28.0	<0.01
3 to <8	95	19.6	175	31.9	
8 to <25	121	24.9	142	25.9	
≥25	179	36.9	78	14.2	
HPV 16 serology					
Negative	340	70.1	491	89.4	<0.01
Positive	145	29.9	58	10.6	

[1]Tests controlled for age and gender.–[2]Data missing on highest level of education for 1 control and 45 cases.

Genotyping for each of the three SNPs was 99% complete. In controls, polymorphisms 194Trp and 280His were rarer than 399Gln (minor allele frequency 6%, 5%, and 35%, respectively). SNPs were in Hardy-Weinberg equilibrium (data not shown). The minor allele frequencies did not differ for whites compared with nonwhites (194Trp, 280His and 399Gln: whites: 0.06, 0.05, 0.35; nonwhites: 0.07, 0.06, 0.32, respectively).

None of the SNPs individually was associated with HNSCC risk (Table II). Each SNP tagged for a unique haplotype (Table III). The most common haplotype contained no variant alleles and served as the referent group for the ORs. The global test on the haplotypes did not indicate an association between *XRCC1* haplotypes and HNSCC risk after controlling for age, sex, race, education, smoking, drinking and HPV16 serology ($p = 0.88$). Further, the ORs for individual haplotypes did not suggest an association.

We examined whether HPV16 serology modified the association between *XRCC1* haplotypes and HNSCC risk (Table IV). The test for interaction was not statistically significant ($p = 0.89$). Next, we investigated an interaction between pack-years smoked and *XRCC1* in HNSCC after stratifying by HPV16 serology. Due to reduced power, we were unable to examine haplotypes among the HPV16 seropositive. Therefore, the *XRCC1* data were simplified to indicate whether a subject had at least one variant allele at any of the loci (Table V). Heavy smoking (≥20 pack-years) modified the relationship between *XRCC1* polymorphisms and HNSCC risk among the HPV16 seronegative ($p_{interaction} = 0.04$) but not among the HPV16 seropositive ($p_{interaction} = 0.89$). For the HPV16 seronegative who were light/never smokers, those with a variant allele were at reduced risk of HNSCC compared with those having no *XRCC1* variants (OR, 0.70; 95% CI, 0.42–1.17); whereas heavy smokers among the HPV16 seronegative were

TABLE II – DISTRIBUTION OF *XRCC1* GENOTYPES AMONG HEAD AND NECK SQUAMOUS CELL CARCINOMA CASES AND CONTROLS

	Genotype	Cases[1] $n = 485$ n (%)	Controls[2] $n = 549$ n (%)	OR[3]	95% CI
Arg194Trp	Arg/Arg	427 (88.2)	485 (88.3)	Referent	
	Arg/Trp	55 (11.4)	61 (11.1)	1.12	(0.72–1.74)
	Trp/Trp	2 (0.4)	3 (0.6)	1.24	(0.19–8.23)
Arg280His	Arg/Arg	437 (90.3)	492 (89.8)	Referent	
	Arg/His	46 (9.5)	52 (9.5)	1.00	(0.62–1.62)
	His/His	1 (0.2)	4 (0.7)	0.29	(0.03–2.70)
Arg399Gln	Arg/Arg	192 (39.8)	232 (42.4)	Referent	
	Arg/Gln	229 (47.4)	246 (45.0)	1.12	(0.83–1.52)
	Gln/Gln	62 (12.8)	69 (12.6)	1.01	(0.65–1.58)

[1]Genotyping unsuccessful for 1 case for Arg194Trp, 1 case for Arg280His, and 2 cases for Arg399Gln.–[2]Genotyping unsuccessful for 1 control for Arg280His and 2 controls for Arg399Gln.–[3]ORs controlled for age, sex, race, education, tobacco, alcohol consumption and HPV16 serology.

TABLE III – ASSOCIATION BETWEEN *XRCC1* HAPLOTYPES AND HEAD AND NECK SQUAMOUS CELL CARCINOMA CASES AND CONTROLS

	Haplotypes			Frequency		OR[1] (95% CI)
	C194T	G280A	G399A	Cases $n = 485$	Controls $n = 549$	
1.	0	0	0	52.5%	53.3%	Referent
2.	0	0	1	36.5%	35.1%	1.04 (0.84–1.29)
3.	1	0	0	6.1%	6.1%	1.14 (0.75–1.72)
4.	0	1	0	4.9%	5.5%	0.91 (0.59–1.42)
Global test on haplotypes, $p = 0.88$						

[1]ORs controlled for age, sex, race, education, tobacco, alcohol consumption and HPV16 serology.

TABLE IV – ASSOCIATION BETWEEN *XRCC1* HAPLOTYPES AND HEAD AND NECK SQUAMOUS CELL CARCINOMA CASES AND CONTROLS, STRATIFIED BY HPV16 SEROLOGY

	Haplotypes			HPV16 Seronegative Frequency		OR[1] (95% CI)	HPV16 Seropositive Frequency		OR[1] (95% CI)
	C194T	G280A	G399A	Cases $n = 340$	Controls $n = 491$		Cases $n = 145$	Controls $n = 58$	
1.	0	0	0	52.5%	53.4%	Referent	52.5%	52.7%	Referent
2.	0	0	1	36.0%	34.9%	1.02 (0.81–1.30)	37.5%	36.9%	1.10 (0.67–1.80)
3.	1	0	0	6.2%	6.2%	1.05 (0.66–1.67)	5.9%	5.2%	1.59 (0.61–4.11)
4.	0	1	0	5.3%	5.5%	0.91 (0.56–1.49)	4.1%	5.2%	0.91 (0.33–2.54)
Test for interaction between haplotypes and HPV16 serology, $p = 0.89$									

[1]ORs controlled for age, sex, race, education, tobacco, and alcohol consumption.

TABLE V – RISK OF HEAD AND NECK SQUAMOUS CELL CARCINOMA FROM COMBINED EXPOSURE TO ANY *XRCC1* POLYMORPHISM AND SMOKING, STRATIFIED BY HPV16 SEROLOGY

HPV16 serology	≥20 Pack-years	*XRCC1* Variant allele[1]	Cases $n = 485$ (%)	Controls $n = 549$ (%)	OR[2]	(95% CI)	$p_{interaction}$, pack-yrs and *XRCC1*
Negative	No	No	38 (7.8)	73 (13.3)	Referent		0.04
		Yes	72 (14.8)	225 (41.0)	0.70	(0.42–1.17)	
	Yes	No	59 (12.2)	64 (11.7)	1.62	(0.90–2.93)	
		Yes	171 (35.3)	129 (23.5)	2.25	(1.35–3.75)	
Positive	No	No	21 (4.3)	9 (1.6)	Referent		0.89
		Yes	51 (10.5)	27 (4.9)	0.84	(0.33–2.14)	
	Yes	No	22 (4.5)	6 (1.1)	1.11	(0.33–3.82)	
		Yes	51 (10.5)	16 (2.9)	1.05	(0.39–2.85)	

[1]Had at least one variant allele in an *XRCC1* polymorphism (194Trp, 280Gln, or 399Gln).–[2]ORs controlled for age, sex, race, education, and alcohol consumption.

more likely to be at an elevated risk of HNSCC. For instance, heavy smoking in the HPV16 seronegative was associated with a nonsignificantly elevated risk of HNSCC among those with no *XRCC1* variants (OR, 1.62; 95% CI, 0.90–2.93) and there was a 2-fold significantly increased risk among heavy smokers with at least one *XRCC1* variant (OR, 2.25; 95% CI, 1.35–3.75). However, among those who were HPV16 seropositive, heavy smoking was not associated with an elevated risk, regardless of *XRCC1* variants.

Though we lacked power to estimate haplotypes among the HPV16 seropositive, we were able to examine *XRCC1* haplotypes for the HPV16 seronegative (Table VI). We again observed that heavy smokers with variant alleles were at greater risk of HNSCC, whereas a variant allele for lighter/never smokers appeared to be associated with a reduced risk of HNSCC, and this difference was of borderline statistical significance ($p_{interaction} = 0.05$). Specifically, heavier smokers (≥20 pack-years) who had the 399Gln haplotype were at increased risk of HNSCC (OR, 1.35; 95% CI, 0.97–1.86). Also, heavier smokers with the 194Trp haplotype had an elevated risk of HNSCC (OR, 1.74; 95% CI, 0.87–3.48). Conversely, among never or light smokers, having a haplotype containing a variant allele appeared to be associated with a reduced

TABLE VI – RESTRICTING TO THE HPV16 SERONEGATIVE: ASSOCIATION BETWEEN *XRCC1* HAPLOTYPES AND HEAD AND NECK SQUAMOUS CELL CARCINOMA CASES AND CONTROLS, STRATIFIED BY PACK-YEARS

	Haplotypes			<20 Pack-years Frequency		OR[1] (95% CI)	≥20 Pack-years Frequency		OR[1] (95% CI)
	C194T	G280A	G399A	Cases $n = 110$	Controls $n = 298$		Cases $n = 230$	Controls $n = 193$	
1.	0	0	0	59.5%	50.8%	Referent	49.1%	57.3%	Referent
2.	0	0	1	29.5%	36.1%	0.74 (0.51–1.08)	39.1%	33.1%	1.35 (0.97–1.86)
3.	1	0	0	6.4%	7.5%	0.65 (0.32–1.33)	6.1%	4.1%	1.74 (0.87–3.48)
4.	0	1	0	4.5%	5.5%	0.69 (0.32–1.48)	5.7%	5.5%	1.10 (0.57–2.13)
Test for interaction between haplotypes and pack-years smoked, $p = 0.05$									

[1]ORs controlled for age, sex, race, education and alcohol consumption.

TABLE VII – *XRCC1* POLYMORPHISMS, SMOKING, AND ASSOCIATION WITH SITE-SPECIFIC HEAD AND NECK SQUAMOUS CELL CARCINOMA, RESTRICTING TO THE HPV16 SERONEGATIVE

≥20 Pack-years	*XRCC1* variant allele[1]	Controls $n = 491$ (%)	Laryngeal Cases $n = 75$ (%)	OR[2]	(95% CI)	Oral Cavity Cases $n = 160$ (%)	OR[2]	(95% CI)	Pharyngeal Cases $n = 103$ (%)	OR[2]	(95% CI)
No	No	73 (14.9)	4 (5.3)	Referent		26 (16.2)	Referent		8 (7.8)	Referent	
	Yes	225 (45.8)	9 (12.0)	0.85	(0.25–2.89)	45 (28.1)	0.64	(0.35–1.16)	17 (16.5)	0.76	(0.31–1.86)
Yes	No	64 (13.0)	19 (25.3)	Referent		21 (13.1)	Referent		19 (18.4)	Referent	
	Yes	129 (26.3)	43 (57.3)	1.08	(0.57–2.03)	68 (42.5)	1.56	(0.85–2.86)	59 (57.3)	1.51	(0.82–2.80)
			$p_{interaction}$	0.73		$p_{interaction}$	0.04		$p_{interaction}$	0.20	

[1]Had at least one variant allele in an *XRCC1* polymorphism (194Trp, 280Gln, or 399Gln).–[2]ORs estimated using a polytomous logistic model that controlled for age, sex, race, education, and alcohol consumption.

risk of HNSCC, and this was of borderline statistical significance for the haplotype containing 399Gln (OR, 0.74; 95% CI, 0.51–1.08).

Next, we examined the interaction between the *XRCC1* SNPs and tobacco use in the HPV16 seronegative by tumor location. There were 93 laryngeal cases, 187 cases of the oral cavity and 203 pharyngeal cases. Of these, 75 laryngeal, 160 oral cavity and 103 pharyngeal cases were HPV16 seronegative (Table VII). Within the strata of never/light smokers who were HPV16 seronegative, having an *XRCC1* polymorphism was associated with a nonsignificantly reduced risk of HNSCC, which was consistent regardless of tumor site. However, among the heavier smokers who were HPV16 seronegative, having an *XRCC1* polymorphism was associated with a nonsignificant 1.5-fold increased risk of HNSCC for tumors of the oral cavity and pharynx. The interaction between pack-years and *XRCC1* polymorphisms reached statistical significance for tumors of the oral cavity ($p_{\text{interaction}} = 0.04$) but not pharyngeal tumors ($p_{\text{interaction}} = 0.20$) due to the reduced power to examine this relationship in the pharynx. We lacked power to examine an interaction between smoking and *XRCC1* polymorphisms by site among the HPV16 seropositive (data not shown).

Discussion

Smoking modified the association between *XRCC1* polymorphisms and HNSCC risk for the HPV16 seronegative but not the HPV16 seropositive. Among the HPV16 seronegative, those who smoked 20 or more pack-years were at increased risk of HNSCC if they carried a variant polymorphism for *XRCC1*. For light or never smokers, who were HPV16 seronegative, *XRCC1* polymorphisms may result in a reduced risk of HNSCC. Among the HPV16 seropositive, there was no relationship between either *XRCC1* polymorphisms or smoking and HNSCC risk. The *XRCC1* polymorphisms tagged for unique haplotypes. The individual haplotypes containing 399Gln or 194Trp were associated with HNSCC risk among heavier smokers without HPV16 exposure. The data suggested that this interaction among the HPV16 seronegative may be most relevant to tumors of the oral cavity and pharynx. However, the site-specific analyses were preliminary as our power to examine this question was low. Overall, we cannot dismiss the role that chance may have had in these results, yet the data further support the literature indicating that HNSCC pathways may differ by HPV status.

Without accounting for exposure to HPV16, the association between *XRCC1* haplotypes, smoking and HNSCC risk would have been missed. That there was no interaction between HPV16 and *XRCC1* is not surprising given that HPV16 carcinogenesis is not directly associated with DNA damage, but rather, the HPV16 proteins E6 and E7 interfere with the tumor suppressors pRb and p53.[41,42] The lack of an interaction between *XRCC1* polymorphisms and HPV16 serology suggests that impaired DNA repair does not influence HPV16 carcinogenesis. Similarly, to our knowledge, no DNA damaging agent has been found to enhance the carcinogenicity of HPV16 in HNSCC. For example, it has been previously reported that smoking, which leads to the formation of DNA adducts, does not further increase risk of HNSCC among the HPV16 positive.[32]

Among the HPV16 seronegative, we found that smoking modified an association between *XRCC1* polymorphisms and HNSCC risk. DNA adducts from smoking are thought to lead to mutations in daughter cells, and these mutations may deactivate tumor suppressors including p53.[43,44] If the *XRCC1* variant alleles result in poorer repair, this increases the likelihood that the DNA adducts would persist, leading to greater risk of HNSCC, consistent with our data among heavy smokers with variant alleles. However, variant alleles for the light/never smokers may be associated with a reduced risk of HNSCC. Previous studies of HNSCC and *XRCC1* polymorphisms have also observed this interaction where the variant allele was associated with a reduced risk in the low exposure category but an elevated risk in the high exposure category, when the exposure was either smoking[18] or alcohol consumption.[21] Researchers, who have studied other types of cancer and found reduced risks associated with *XRCC1* polymorphisms, have attributed this to persistent DNA damage in cells containing the variant allele which leads to more mutations, making the cell more likely to undergo apoptosis.[45–47] On the other hand, heavy smokers with variant alleles thus leading to reduced repair capacity may harbor a greater burden of mutations and an increased likelihood of compromising genes involved in apoptosis (*e.g.*, the deactivation of p53), thereby preventing cell death. Thus, the cell and the DNA damage persist, resulting in greater HNSCC risk.

Our estimation of *XRCC1* haplotypes and their distribution was similar to other studies.[15,19] In addition, our observation of no overall association between HNSCC risk and *XRCC1* polymorphisms was consistent with previous research,[15,18,19,23] as was the finding of an interaction with smoking.[16,18,22] However, as stated earlier, not all previous studies were in agreement with these results. Our results indicate that the discrepancies in the literature may be due to differences in the distribution of smoking and exposure to HPV16 across populations, and that consideration of HPV16 status may be necessary to appropriately evaluate the smoking-*XRCC1* relationship in HNSCC.

The allele frequencies we observed were comparable to the frequencies reported in other white populations in the United States,[18,21–23] however, reported frequencies for the 194Trp and 280His alleles tended to be slightly higher and the 399Gln allele frequency lower in Korean[15] and Indian[17,48] populations. The majority of the nonwhites in our study population were black, and their allele frequencies did not vary greatly from that of whites. This similarity has been reported in studies of *XRCC1* where both groups were represented,[18,49] although not always.[50] When we compared results that included subjects from all backgrounds and controlled for race with models that restricted to whites, the results were unchanged.

The use of serology to determine exposure to HPV16 has its limitations. For example, serology is not site-specific. Thus, being seropositive is not indicative of where in the body infection occurred, although previously we have demonstrated that increasing HPV titer was associated with presence of HPV DNA in HNSCC tumors.[35] In addition, HPV serology has been found to have strong specificity but weaker sensitivity.[51] In particular, investigators have reported that seroconversion is less likely with transient infections than with persistent ones.[52] As a result, it is possible that we are underestimating past HPV16 exposure. Potential misclassification of HPV16 exposure may have reduced the observed associations, although we cannot say with certainty what impact this misclassification may have had on the observed associations and interactions.

A limitation of this study was the lower participation rate in the controls, a widespread challenge in population-based case-control studies. Among those who did participate, the majority also provided blood samples from which both *XRCC1* genotypes and HPV16 seropositivity were determined. Thus, for bias to influence our results, controls would have had to preferentially participate by *XRCC1* genotype or by HPV16 exposure. This appears unlikely given that subjects were not informed of the study hypotheses. In contrast, smoking was self-reported, and it has been reported that subjects who decreased their smoking tended to underestimate their past cigarette consumption.[53] Therefore, if cases had recently decreased their smoking due to disease, it is possible that they may under-report their past cigarette use. If this was the case, the association with smoking may be underestimated.

XRCC1 polymorphisms may confer susceptibility to HNSCC in the context of smoking, among those who are HPV16 seronegative. Our analysis provides support to the growing recognition that HPV16-related HNSCC is a distinct disease and future research into HNSCC susceptibility and other HNSCC risk factors should examine their disease impact separately by HPV16 status.

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