

Selected Genetic Polymorphisms in *MGMT*, *XRCC1*, *XPD*, and *XRCC3* and Risk of Head and Neck Cancer: A Pooled Analysis

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Abstract

Tobacco and alcohol consumption are the major risk factors for head and neck cancer, likely due to DNA-damaging processes. Genetic variations in DNA repair genes may affect an individual's susceptibility to head and neck cancer. Pooling data and DNA specimens from three case-control studies in western Washington State, North Carolina, and Puerto Rico, totaling 555 cases (430 whites) and 792 controls (695 whites), we studied the risk of head and neck cancer in relation to common nonsynonymous single-nucleotide polymorphisms in four DNA repair genes: *MGMT* (*Leu₈₄Phe* and *Ile₁₄₃Val*), *XRCC1* (*Arg₃₉₉Gln*), *XPD* (*Lys₇₅₁Gln*), and *XRCC3* (*Thr₂₄₁Met*). All single-nucleotide polymorphisms were assayed in a single laboratory. Among whites, carriage of the *MGMT Phe₈₄* [odds ratio (OR), 0.71; 95% confidence

interval (95% CI), 0.51-0.98] or *Val₁₄₃* (OR, 0.66; 95% CI, 0.47-0.92) allele was associated with a decreased risk of head and neck cancer; the haplotype distribution for *MGMT* differed significantly between cases and controls (covariate-adjusted global permutation test, $P = 0.012$). The *XRCC1 GlnGln₃₉₉* genotype was also associated with decreased risk among whites (OR, 0.56; 95% CI, 0.32-0.94), whereas *XPD₇₅₁* and *XRCC3₂₄₁* were not associated with risk. Alcohol-related risks tended to vary with DNA repair genotypes, especially for *MGMT* variants, whereas no effect modification was noted with tobacco use. Consistent findings from three case-control studies suggest that selected DNA repair enzymes may play a role in head and neck carcinogenesis. (Cancer Epidemiol Biomarkers Prev 2005;14(7):1747-53)

Introduction

Tobacco and alcohol account for more than 75% of squamous cell head and neck cancer (oral, pharyngeal, and laryngeal cancer; ref. 1, 2), but specific carcinogenic mechanisms are unclear. Genetic factors are likely to play a role in head and neck cancer because only a small proportion of heavy tobacco and alcohol users develop this disease and the risk of head and neck cancer is higher among first-degree relatives of head and neck cancer cases, even after adjustment for smoking and alcohol (3). Metabolites of tobacco (4, 5) and alcohol (6-8) cause DNA damage by producing oxidative stress, alkylation, bulky adducts, and strand breaks. Altered DNA repair capacity may increase the risk of various cancers, including head and neck cancer (9-11).

There are several known DNA repair pathways, providing distinct but overlapping protection against mutagenetic exposures. The base excision repair pathway is involved in the removal of simple base modifications and oxidative DNA damage, such as single-strand breaks, nonbulky adducts, and alkylation adducts (12). The X-ray cross-complementing group 1 (*XRCC1*) gene product acts as a scaffold protein and coordinates the actions of polymerase β , DNA ligase III, and poly(ADP-ribose) polymerase in short-patch base excision repair (13). The *XRCC1 Arg₃₉₉Gln* polymorphism is located in an evolutionarily conserved region of the gene and is

hypothesized to alter the function of *XRCC1* (14, 15). The nucleotide excision repair pathway primarily removes and repairs bulky adducts, but has been reported to play a role in repair of oxidative DNA damage as well (16, 17). The xeroderma pigmentosum group D (*XPD*; originally named excision repair cross complementing group 2) protein, a subunit of transcription factor IIIH, is an evolutionarily conserved 5' \rightarrow 3' helicase that unwinds the DNA in the region of DNA damage. The *Gln₇₅₁* variant, being located about 50 bases upstream from the poly(A) site, is suspected to alter *XPD* protein function (18), but functional results have been inconsistent (14, 19). The homologous recombination pathway repairs double-strand DNA breaks in the S-G₂ phases of the cell cycle (20). The role of *XRCC3* in homologous recombination is not entirely clear, however, it interacts with Rad51 (21), which catalyzes DNA strand exchange in homologous recombination, and *XRCC3*-deficient cell lines display reduced homologous recombination repair (22). The *XRCC3 Met₂₄₁* variant was significantly associated with higher DNA adduct levels (23) and homology-directed repair activity (24). *O*⁶-Methylguanine-DNA methyltransferase (*MGMT*, also named *O*⁶-alkylguanine-DNA alkyltransferase), is the principal mechanism for repairing *O*⁶-alkylguanine adducts (25). The alkyltransferase binds to and removes alkyl groups from the *O*⁶ position of guanine in a single step. Both the *MGMT* codon 84 and 143 variants are evolutionarily conserved (26, 27) and the *MGMT₁₄₃* polymorphism is close to the *Cys₁₄₅* alkyl acceptor site (26), but functional importance of either variant is unknown (25, 28).

It is unclear which DNA repair pathways or enzymes may be most important for protection against head and neck cancer. Previous studies suggested that single nucleotide polymorphisms (SNP) in *XRCC1* and *XPD* may be associated with

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head and neck cancer risk (29–32), but the findings have been inconsistent (29, 30, 32). To clarify the role of *XRCC1* and *XPB* polymorphisms and to explore the role of other DNA repair pathways in susceptibility to head and neck cancer, we studied the risk of head and neck cancer in relationship to common amino acid substitution (nonsynonymous) SNPs in four DNA repair genes, *XRCC1* (*Arg₃₉₉Gln*), *XPB* (*Lys₇₅₁Gln*), *MGMT* (*Leu₈₄Phe* and *Ile₁₄₃Val*), and *XRCC3* (*Thr₂₄₁Met*), in a pooled analysis of 555 cases and 792 controls, from three case-control studies.

Materials and Methods

Study Populations. The Washington Study is an aggregate of two population-based, case-control studies (33) conducted among western Washington state residents, including 407 cases with cancer of the oral cavity and pharynx and 615 controls. Controls were selected by random-digit telephone dialing, frequency-matched to the cases by age and sex. DNA was extracted from exfoliated buccal cells or venous blood for 92% of interviewed subjects (365 cases and 576 controls). The North Carolina Study is a hospital-based, case-control study (34) of 182 cases of squamous cell carcinoma of the oral cavity, pharynx, and larynx and 202 controls, frequency-matched to cases by age and gender. DNA was derived from blood or buccal swab samples for 97% of interviewed subjects (176 cases and 195 controls). Samples from this study were previously genotyped for *XRCC1₃₉₉* (29); however, all samples were reassayed for the pooled analysis. The Puerto Rico Study is a population-based, case-control study with 342 cases of oral and pharyngeal cancer and 521 controls frequency-matched to cases by age (35). DNA was extracted from buccal cell specimens for 52% of subjects eligible for sample collection (137 cases and 146 controls).

Genotyping. All samples were genotyped at the National Cancer Institute Core Genotyping Facility, using matrix-assisted laser desorption/ionization time-of-flight mass spec-

trometry (36) for the Washington Study samples and TaqMan (37) for the other samples (<http://snp500cancer.nci.nih.gov>). Internal laboratory quality controls consisted of Coriell DNA samples representing four of each genotype (homozygous major allele, heterozygous, and homozygous minor allele) for each polymorphism and four no template controls, in every 384 samples. External blinded quality controls (i.e., 89 duplicate or triplicate samples from 35 individuals) were also used for each polymorphism, showing ≥97% concordance for all assays except *XRCC3₂₄₁* (95% concordance).

Statistical Analysis. Departures from Hardy-Weinberg equilibrium were assessed among controls by race and study. Odds ratios (OR) and 95% confidence intervals (CI) were calculated by unconditional logistic regression, adjusting for gender, race, age, lifetime average use of smoking and alcohol, and study center (for pooled analyses). Random effect models were used to estimate the pooled ORs and 95% CIs for all five SNPs (*P* for heterogeneity: 0.9 for *MGMT₈₄*, 0.7 for *MGMT₁₄₃*, 0.2 for *XRCC1₃₉₉*, 0.06 for *XPB₇₅₁*, and 1.0 for *XRCC3₂₄₁*). Departures from the multiplicative benchmark for the interaction between genotype and exposure (e.g., smoking or alcohol) were assessed by comparing nested models with and without cross-product terms using a likelihood ratio test. Head and neck cancer risks associated with haplotypes defined by the *MGMT* SNPs were assessed using HaploStats (<http://www.mayo.edu/hsr/people/schaid.html>), employing the expectation-maximization algorithm to estimate haplotypes and a global score test to assess overall differences in haplotype frequencies between cases and controls, adjusted for covariates (38, 39). Haplotype-associated risks were assessed for each study using the generalized linear model implemented in HaploStats and for the pooled analysis using random effect models.

Results

Selected characteristics of the subjects in the three studies are displayed in Table 1. Most subjects were white (77% of cases and

Table 1. Selected characteristics of the study populations

	Washington		North Carolina		Puerto Rico		Pooled	
	Population based		Hospital based		Population based		Cases, [‡] n = 555	Controls, n = 792
	Cases,* n = 279	Controls, n = 472	Cases, [†] n = 159	Controls, n = 183	Cases, [‡] n = 117	Controls, n = 137		
Race, n (%)								
White	259 (93)	443 (94)	94 (59)	159 (87)	77 (66)	93 (68)	430 (77)	695 (88)
Black	11 (4)	14 (3)	60 (38)	22 (12)	12 (10)	10 (7)	83 (15)	46 (6)
Others	9 (3)	15 (3)	5 (3)	2 (1)	28 (24)	34 (25)	42 (8)	51 (6)
Gender, n (%)								
Male	199 (71)	332 (70)	125 (79)	102 (56)	105 (90)	107 (78)	429 (77)	541 (68)
Female	80 (29)	140 (30)	34 (21)	81 (44)	12 (10)	30 (22)	126 (23)	251 (32)
Education, n (%)								
<High school (<12 y)	9 (4)	12 (3)	83 (52)	33 (18)	85 (73)	89 (65)	177 (33)	134 (17)
High school graduate (12 y)	101 (40)	125 (26)	38 (24)	51 (28)	18 (15)	14 (10)	157 (30)	190 (24)
Technical school	16 (6)	28 (6)	5 (3)	10 (6)	4 (3)	9 (7)	25 (5)	47 (6)
College	101 (40)	232 (49)	12 (8)	26 (14)	3 (3)	8 (6)	116 (22)	266 (33)
Graduate school	24 (10)	75 (16)	21 (13)	63 (34)	7 (6)	17 (12)	52 (10)	155 (20)
Age (y)								
Mean (SD)	56.0 (8.7)	55.0 (9.6)	60.0 (12.0)	58.0 (12.4)	65.0 (9.5)	67.0 (10.9)	58.0 (10.6)	58.0 (11.4)
Smoking								
Cigarettes/d	20.0 (15.5)	10.0 (15.7)	20.0 (14.2)	8.0 (17.1)	20.0 (19.3)	2.0 (16.2)	20.0 (16.1)	8.0 (16.2)
Total years of smoking	33.0 (16.9)	13.0 (16.0)	35.0 (16.1)	6.0 (16.6)	38.0 (19.3)	4.0 (19.5)	35.0 (17.3)	10.0 (16.8)
Alcohol								
Drinks/wk	11.8 (36.0)	3.7 (15.8)	20.9 (67.4)	1.0 (44.9)	60.0 (71.2)	4.4 (26.8)	19.0 (58.5)	3.5 (27.2)
Total years of drinking	—	—	30.0 (17.2)	3.0 (16.7)	38.0 (16.6)	31.0 (21.2)	—	—

NOTE: Data expressed as n (%) or median (SD).

*Cancers of tongue, gum, mouth floor, tonsils, and oropharynx.

†Cancers of oral cavity, pharynx, and larynx.

‡Cancers of oral cavity (excluding lip and salivary glands) and pharynx (excluding nasopharynx).

Table 2. Pooled analysis of head and neck cancer risk associated with smoking, alcohol, and selected DNA repair genotypes

	All subjects		White subjects	
	Cases = 555, controls = 792		Cases = 430, controls = 695	
	<i>n</i> * (case, control)	OR (95% CI) [†]	<i>n</i> * (case, control)	OR (95% CI) [†]
Smoking				
Never	74, 309	1.0	67, 274	1.0
1-20 cigarettes/d	232, 316	2.23 (1.61-3.09)	167, 268	1.99 (1.40-2.82)
≥21 cigarettes/d	214, 163	3.31 (2.30-4.77)	167, 150	3.00 (2.04-4.39)
<i>P</i> _{trend}		<0.001		<0.001
Alcohol				
Never or <1 drink/wk	77, 263	1.0	70, 233	1.0
1-20 drinks/wk	185, 409	1.39 (0.98-1.96)	160, 372	1.29 (0.90-1.85)
≥21 drinks/wk	248, 111	5.58 (3.69-8.44)	167, 86	5.01 (3.21-7.81)
<i>P</i> _{trend}		<0.001		<0.001
MGMT ₈₄				
LeuLeu	386, 529	1.0	315, 468	1.0
LeuPhe	117, 204	0.75 (0.56-1.02)	80, 179	0.72 (0.52-1.01)
PhePhe	11, 21	0.64 (0.26-1.60)	5, 18	0.41 (0.12-1.17) [‡]
LeuPhe+PhePhe		0.74 (0.55-1.00)		0.71 (0.51-0.98)
<i>P</i> _{trend}		0.05		0.03
MGMT ₁₄₃				
IleIle	434, 570	1.0	325, 488	1.0
IleVal	96, 180	0.72 (0.52-0.99)	81, 172	0.64 (0.45-0.90)
ValVal	6, 12	0.66 (0.20-1.91) [‡]	6, 12	0.75 (0.23-2.19) [‡]
IleVal+ValVal		0.73 (0.53-1.00)		0.66 (0.47-0.92)
<i>P</i> _{trend}		0.08		0.03
XRCC1 ₃₉₉				
ArgArg	266, 338	1.0	187, 283	1.0
ArgGln	219, 338	0.91 (0.66-1.25)	184, 306	0.97 (0.73-1.30)
GlnGln	40, 81	0.40 (0.11-1.51)	33, 75	0.56 (0.32-0.94)
ArgGln+GlnGln		0.84 (0.65-1.09)		0.89 (0.67-1.17)
<i>P</i> _{trend}		0.11		0.10
XPD ₇₅₁				
LysLys	240, 345	1.0	176, 296	1.0
LysGln	235, 325	1.04 (0.80-1.37)	188, 292	1.07 (0.80-1.44)
GlnGln	69, 105	1.03 (0.69-1.52)	61, 95	1.31 (0.70-2.43)
LysGln+GlnGln		1.04 (0.81-1.34)		1.10 (0.83-1.45)
<i>P</i> _{trend}		0.82		0.49
XRCC3 ₂₄₁				
ThrThr	232, 329	1.0	159, 267	1.0
ThrMet	223, 334	1.01 (0.76-1.33)	181, 309	0.98 (0.72-1.32)
MetMet	61, 97	1.15 (0.76-1.74)	54, 90	1.16 (0.75-1.80)
ThrMet+MetMet		1.04 (0.80-1.35)		1.02 (0.76-1.35)
<i>P</i> _{trend}		0.60		0.64

**n*: pooled from Washington Study (279 cases and 472 controls), North Carolina Study (159 cases and 183 controls), and Puerto Rico Study (117 cases and 137 controls); numbers do not add up to the column totals due to missing values.

[†]Estimated using a random effect model adjusted for gender, race, age, smoking, alcohol use, and center.

[‡]Exact estimate and 95% CI.

88% of controls), male (77% of cases and 68% of controls), and >55 years of age (median age: cases, 59; controls, 58). Cigarette smoking and alcohol drinking were associated with increased risks of head and neck cancer in all three studies (data not shown), as well as in the pooled analysis (Table 2). Genotype distributions among controls were consistent with Hardy-Weinberg equilibrium in each study, and overall for whites, blacks, and other racial groups ($P > 0.05$).

Among whites, carriage of the MGMT *Phe*₈₄ allele or the MGMT *Val*₁₄₃ allele was associated with decreased risk for head and neck cancer in all three studies (Fig. 1) and in the pooled analysis [OR, 0.71 (95% CI, 0.51-0.98) and OR, 0.66 (95% CI, 0.47-0.92), respectively]; similar associations were found for all ethnic groups combined (Table 2). The two MGMT SNPs were weakly linked ($D' = 0.31$), and adjustment of one for the other led to comparable results. The genotype-based analysis for MGMT alleles is supported by the haplotype analysis showing different distributions for MGMT between cases and controls (pooled global permutation test: $P_{\text{adjusted}} = 0.01$ for whites only, and $P_{\text{adjusted}} = 0.05$ for all subjects combined). A similar reduction in risk was found for each of the MGMT haplotypes containing only one of the

low-risk variants compared with the *Leu*₈₄-*Ile*₁₄₃ haplotype (data not shown). However, the haplotype containing both low-risk alleles was too rare (1%) to yield a precise estimate of risk.

Among whites, XRCC1 *Gln*₃₉₉ homozygotes were associated with a decreased risk of head and neck cancer compared with wild-type homozygotes in all three studies (Fig. 1), as well as the pooled analysis (OR, 0.56; 95% CI, 0.32-0.94). No independent associations were found for XPD₇₅₁ or XRCC3₂₄₁. Exclusion of the laryngeal cancers ($n = 48$) did not materially alter any of the results (data not shown).

Alcohol-related head and neck cancer risks tended to be less pronounced among carriers of MGMT *Val*₁₄₃, XPD *Gln*₇₅₁, or the XRCC3 *Met*₂₄₁ allele (Table 3; P for interaction for all subjects: 0.06, 0.02, and 0.006, respectively, and for whites: 0.1, 0.02, and 0.008, respectively). For example, among whites who drank ≥21 drinks per week, carriage of MGMT *Val*₁₄₃ allele was associated with a decreased risk (OR, 0.4; 95% CI, 0.2-0.8), whereas no clear association was found for light drinkers and abstainers; similar patterns were also found for all ethnic groups combined. Smoking-related risks did not vary substantially by genotype (data not shown).

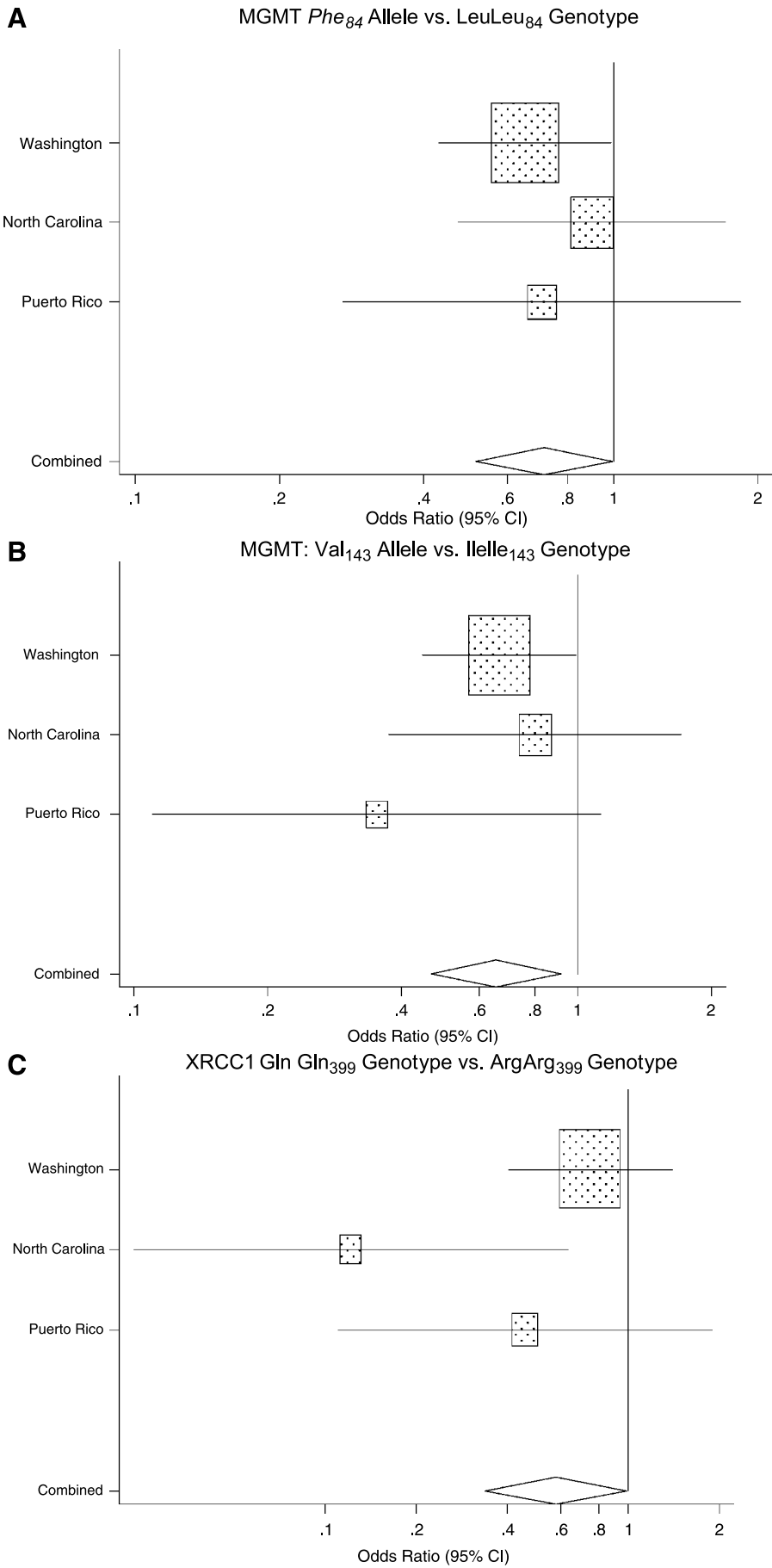


Figure 1. Results of the association between head and neck cancer and *MGMT* and *XRCC1* genotypes in individual studies and pooled analyses. ORs and 95% CIs shown are for white subjects only, adjusted for gender, age, smoking, alcohol, and center (for pooled analysis).

Table 3. Pooled analysis of head and neck cancer risk associated with the joint effect of alcohol use and DNA repair genotypes

	Alcohol [OR* (95% CI); n (case, control)]							
	All subjects				White subjects			
	Never or <1 drink/wk	1-20 drinks/wk	≥21 drinks/wk	<i>P</i> _{trend}	Never or <1 drink/wk	1-20 drinks/wk	≥21 drinks/wk	<i>P</i> _{trend}
<i>MGMT</i> ₈₄								
<i>LeuLeu</i>	1.0	1.3 (0.9-1.9)	4.6 (2.8-7.4)	<0.001	1.0	1.2 (0.8-1.9)	4.1 (2.5-6.9)	<0.001
	54, 165	137, 280	163, 80		50/146	121/254	116/66	
<i>PhePhe/LeuPhe</i>	0.6 (0.3-1.2)	0.9 (0.5-1.5)	4.9 (2.6-9.3)	<0.001	0.7 (0.3-1.3)	0.8 (0.5-1.3)	4.1 (2.0-8.5)	<0.001
	17, 79	39, 119	61, 23		15/70	32/109	35/17	
<i>P</i>	0.2	0.1	0.8	<i>P</i> _{interaction} = 0.2	0.2	0.05	1.0	<i>P</i> _{interaction} = 0.4
<i>MGMT</i> ₁₄₃								
<i>IleIle</i>	1.0	1.4 (0.9-2.0)	6.4 (4.0-10.2)	<0.001	1.0	1.3 (0.9-1.9)	5.6 (3.4-9.4)	<0.001
	59, 194	139, 295	202, 74		53/169	119/260	128/56	
<i>ValVal/IleVal</i>	1.0 (0.5-1.8)	1.2 (0.7-2.0)	2.7 (1.5-5.1)	0.007	0.9 (0.5-1.7)	1.0 (0.6-1.7)	2.4 (1.2-4.8)	<0.001
	17, 59	38, 101	36, 30		16/55	34/101	29/27	
<i>P</i>	0.9	0.5	0.004	<i>P</i> _{interaction} = 0.06	0.7	0.2	0.01	<i>P</i> _{interaction} = 0.1
<i>XRCC1</i> ₃₉₉								
<i>ArgArg</i>	1.0	1.2 (0.8-2.0)	4.5 (2.6-7.9)	<0.001	1.0	1.1 (0.7-1.9)	3.6 (2.0-6.7)	<0.001
	38, 107	83, 171	122, 55		34/91	68/150	70/40	
<i>GlnGln/ArgGln</i>	0.7 (0.4-1.1)	1.0 (0.6-1.7)	4.6 (2.6-8.1)	<0.001	0.7 (0.4-1.2)	0.9 (0.6-1.6)	4.3 (2.4-7.8)	<0.001
	32, 141	92, 222	114, 52		31/129	82/207	87/43	
<i>P</i>	0.1	0.4	0.9	<i>P</i> _{interaction} = 0.3	0.1	0.4	0.5	<i>P</i> _{interaction} = 0.1
<i>XPB</i> ₇₅₁								
<i>LysLys</i>	1.0	1.9 (1.1-3.2)	9.2 (5.0-17.0)	<0.001	1.0	1.8 (1.0-3.2)	8.5 (4.3-16.7)	<0.001
	26, 120	75, 176	119, 44		22/105	63/156	79/34	
<i>GlnGln/LysGln</i>	1.8 (1.0-3.0)	2.0 (1.2-3.4)	7.1 (4.0-12.7)	<0.001	1.9 (1.1-3.4)	2.0 (1.1-3.5)	6.9 (3.7-12.9)	<0.001
	49, 137	107, 227	123, 62		47/124	94/210	87/50	
<i>P</i>	0.05	0.7	0.3	<i>P</i> _{interaction} = 0.02	0.03	0.7	0.5	<i>P</i> _{interaction} = 0.02
<i>XRCC3</i> ₂₄₁								
<i>ThrThr</i>	1.0	2.4 (1.4-4.3)	11.4 (5.9-21.9)	<0.001	1.0	2.2 (1.2-4.1)	11.1 (5.3-23.2)	<0.001
	20, 116	79, 164	118, 44		17/96	63/141	68/28	
<i>MetMet/ThrMet</i>	2.1 (1.2-3.7)	2.1 (1.2-3.7)	8.3 (4.5-15.4)	<0.001	1.9 (1.0-3.6)	1.9 (1.0-3.5)	7.1 (3.6-13.8)	<0.001
	49, 138	89, 228	118, 61		45/128	81/216	88/53	
<i>P</i>	0.02	0.4	0.2	<i>P</i> _{interaction} = 0.006	0.04	0.5	0.1	<i>P</i> _{interaction} = 0.008

*Adjusted for gender, race, age, smoking, and center.

Discussion

Consistent results from three case-control studies and a pooled analysis, totaling 555 head and neck cancer cases and 792 controls, suggest that genetic variations in *MGMT*₈₄, *MGMT*₁₄₃, and *XRCC1*₃₉₉ influence susceptibility to head and neck cancer. Moreover, the *MGMT*₁₄₃ variant may modify alcohol-related risk.

MGMT encodes *O*⁶-alkylguanine DNA alkyltransferase, which preferentially removes *O*⁶-guanine alkyl adducts caused by carcinogens, such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone found in tobacco smoke (40), and the *Val*₁₄₃ allele has previously been related to increased lung cancer risk in two small studies (each with ~130 cases; refs. 28, 41). In our study, *Val*₁₄₃ was associated with reduced head and neck cancer risk, particularly among heavy drinkers. *In vivo* and *in vitro* experiments show that *MGMT*-mediated repair of alkylated DNA is reduced by treatment with ethanol or its primary metabolite, acetaldehyde (42, 43), possibly due to *MGMT* inhibition (44). Although the functional importance of either the *MGMT* codon 84 or 143 variants is unknown (25, 28), both are evolutionarily conserved (26, 27) and the *MGMT*₁₄₃ polymorphism is close to the *Cys*₁₄₅ alkyl acceptor site (26). Observed associations may also be due to linkage with other functional variants (45), such as the *MGMT* variant in the promoter-enhancer region found to be associated with increased *MGMT* activity in cell lines (46).

We found a consistently decreased risk of head and neck cancer in the three studies for the *XRCC1 Gln*₃₉₉ homozygote, in comparison with a marginally increased risk and no association reported in smaller studies of head and neck cancer among U.S. whites (30) and Koreans (with approxi-

mately 203 and 147 cases, respectively; ref. 32). Functional data do not help clarify this: the *Gln*₃₉₉ variant has been associated with excess DNA damage (14, 15), increased *p53* mutations (47), and reduced DNA capacity (48); in other studies, no effect was noted on DNA repair capacity (49) and a nonsignificant reduction in DNA adduct levels was found among smokers (23). Possible explanations for the discordance of findings include the following: The sample sizes in these functional studies were generally small making the estimates unstable. The effect of the *XRCC1* variant on DNA repair capacity may differ with type and strength of the DNA damaging exposures. The studied variant in association with reduced head and neck cancer risk may be in linkage with other unidentified functional variants that account for increased cancer risk. Also, cells with reduced DNA capacity may undergo apoptosis instead of repair if there is extensive DNA damage. Alternatively, some of these results may be chance findings.

We observed more heterogeneity in results across the three studies for the *XPB Gln*₇₅₁ variant (no associations in the Washington and Puerto Rico studies and an increased risk in the North Carolina study) yielding no overall association with head and neck cancer. This was not consistent with a marginally increased risk previously reported (189 head and neck cancer cases; ref. 31). The *LysLys*₇₅₁ genotype was associated with higher number of chromatid aberrations (19), but not with polyphenol DNA adducts (14). We found no effect with the *XRCC3 Thr*₂₄₁*Met* polymorphism, consistent with results from a French study of 121 oral/pharynx and 129 larynx cancer cases (50). We are less convinced of the statistical interactions seen between alcohol use and the *XPB*₇₅₁ and *XRCC3*₂₄₁ polymorphisms because of lack of an independent

main effect for the genotype, lack of biological support for the association, and the heterogeneous results of *XPD*₇₅₁ between the studies.

Based on a study of selected genes and SNPs, we found that *MGMT* and perhaps *XRCC1* may be important in head and neck carcinogenesis, but potential roles by other DNA repair genes not evaluated cannot be ruled out. Also, although our study had a relatively large sample size, interaction ORs were imprecise and the role of chance cannot be dismissed. Future studies on exposure-specific (e.g., alcohol) and tumor tissue-specific expression patterns (as opposed to lymphocytes as a surrogate), evaluated in the context of a better characterized gene haplotype structure (rather than one SNP at a time), may help advance our understanding. Future large epidemiologic studies to replicate our results on these SNPs and to explore other DNA repair genes and SNPs are also needed.

This is the first report to show that *MGMT* polymorphisms are associated with head and neck cancer risk, as shown in three separate geographic regions. Further epidemiologic studies are needed to clarify the effects of *MGMT* and other DNA repair genes in head and neck cancer risk, and to elaborate interactions with alcohol and other exposures.

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