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

Wen-Yi Huang,[1] Andrew F. Olshan,[3] Stephen M. Schwartz,[4,5] Sonja I. Berndt,[1] Chu Chen,[5] Victor Llaca,[6] Stephen J. Chanock,[1,2] Joseph F. Fraumeni, Jr.,[1] and Richard B. Hayes[1]

[1]Division of Cancer Epidemiology and Genetics and [2]Pediatric Oncology Branch, National Cancer Institute, NIH, Department of Health and Human Services, Bethesda, Maryland; [3]Department of Epidemiology, School of Public Health, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina; [4]Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, Washington; [5]Department of Epidemiology, University of Washington, Seattle, Washington, and [6]Core Genotyping Facility, Advanced Technology Center, National Cancer Institute-Frederick, Gaithersburg, Maryland

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* (*$Leu_{84}Phe$* and *$Ile_{143}Val$*), *XRCC1* (*$Arg_{399}Gln$*), *XPD* (*$Lys_{751}Gln$*), and *XRCC3* (*$Thr_{241}Met$*). All single-nucleotide polymorphisms were assayed in a single laboratory. Among whites, carriage of the *MGMT* *Phe_{84}* [odds ratio (OR), 0.71; 95% confidence interval (95% CI), 0.51-0.98] or Val_{143} (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* $GlnGln_{399}$ genotype was also associated with decreased risk among whites (OR, 0.56; 95% CI, 0.32-0.94), whereas *XPD_{751}* and *$XRCC3_{241}$* 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* *$Arg_{399}Gln$* 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 IIH, is an evolutionarily conserved 5′ →3′ helicase that unwinds the DNA in the region of DNA damage. The Gln_{751} 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-G_2 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* Met_{241} 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 $MGMT_{143}$ polymorphism is close to the *Cys_{145}* 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

Received 3/14/05; revised 4/19/05; accepted 4/5/05.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Wen-Yi Huang, Division of Cancer Epidemiology and Genetics, National Cancer Institute, EPS 8113, MSC 7240, Bethesda, MD 20892. Phone: 301-435-4710; Fax: 301-402-1819. E-mail: huangw@mail.nih.gov

head and neck cancer risk (29–32), but the findings have been inconsistent (29, 30, 32). To clarify the role of *XRCC1* and *XPD* 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* ($Arg_{399}Gln$), *XPD* ($Lys_{751}Gln$), *MGMT* ($Leu_{84}Phe$ and $Ile_{143}Val$), and *XRCC3* ($Thr_{241}Met$), 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 $XRCC1_{399}$ (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 spectrometry (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 $XRCC3_{241}$ (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 $MGMT_{84}$, 0.7 for $MGMT_{143}$, 0.2 for $XRCC1_{399}$, 0.06 for XPD_{751}, and 1.0 for $XRCC3_{241}$). 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 = 279	Controls, n = 472	Cases,† n = 159	Controls, n = 183	Cases,‡ n = 117	Controls, n = 137	Cases,‡ n = 555	Controls, n = 792
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	
	n* (case, control)	OR (95% CI)†	n* (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)
P_{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)
P_{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)
P_{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)
P_{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)
P_{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)
P_{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)
P_{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: $P_{adjusted} = 0.01$ for whites only, and $P_{adjusted} = 0.05$ 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 XPD_{751} or $XRCC3_{241}$. 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).

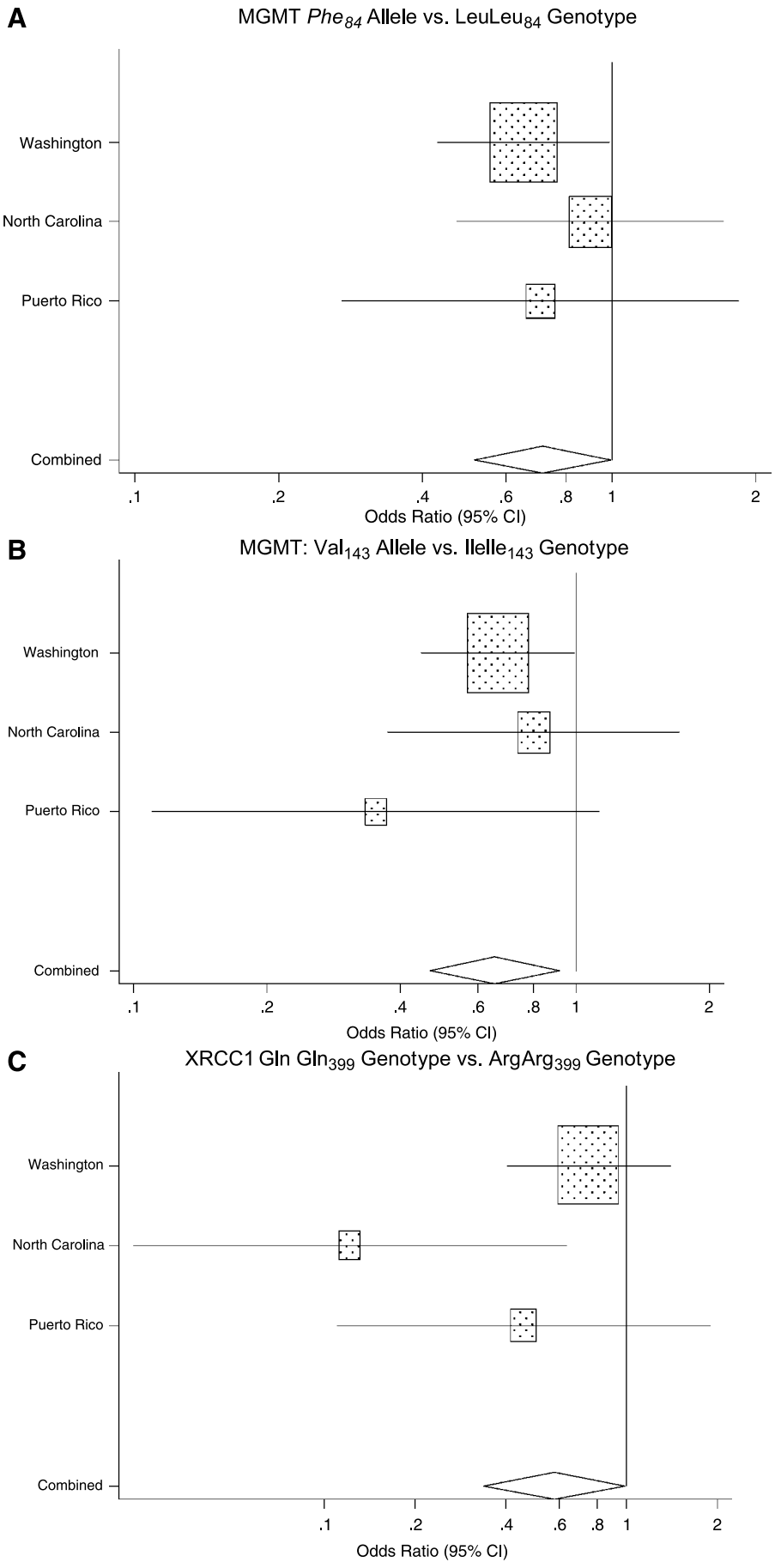

Figure 1. Results of the association between head and neck cancer and *MGMT* and *XRCC1* genotypes in individual studies and pooled analyses. ORs and 95% CIs shown are for white subjects only, adjusted for gender, age, smoking, alcohol, and center (for pooled analysis).

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	P_{trend}	Never or <1 drink/wk	1-20 drinks/wk	≥21 drinks/wk	P_{trend}
$MGMT_{84}$								
LeuLeu	1.0 54, 165	1.3 (0.9-1.9) 137, 280	4.6 (2.8-7.4) 163, 80	<0.001	1.0 50/146	1.2 (0.8-1.9) 121/254	4.1 (2.5-6.9) 116/66	<0.001
PhePhe/LeuPhe	0.6 (0.3-1.2) 17, 79	0.9 (0.5-1.5) 39, 119	4.9 (2.6-9.3) 61, 23	<0.001	0.7 (0.3-1.3) 15/70	0.8 (0.5-1.3) 32/109	4.1 (2.0-8.5) 35/17	<0.001
P	0.2	0.1	0.8	$P_{interaction} = 0.2$	0.2	0.05	1.0	$P_{interaction} = 0.4$
$MGMT_{143}$								
IleIle	1.0 59, 194	1.4 (0.9-2.0) 139, 295	6.4 (4.0-10.2) 202, 74	<0.001	1.0 53/169	1.3 (0.9-1.9) 119/260	5.6 (3.4-9.4) 128/56	<0.001
ValVal/IleVal	1.0 (0.5-1.8) 17, 59	1.2 (0.7-2.0) 38, 101	2.7 (1.5-5.1) 36, 30	0.007	0.9 (0.5-1.7) 16/55	1.0 (0.6-1.7) 34/101	2.4 (1.2-4.8) 29/27	<0.001
P	0.9	0.5	0.004	$P_{interaction} = 0.06$	0.7	0.2	0.01	$P_{interaction} = 0.1$
$XRCC1_{399}$								
ArgArg	1.0 38, 107	1.2 (0.8-2.0) 83, 171	4.5 (2.6-7.9) 122, 55	<0.001	1.0 34/91	1.1 (0.7-1.9) 68/150	3.6 (2.0-6.7) 70/40	<0.001
GlnGln/ArgGln	0.7 (0.4-1.1) 32, 141	1.0 (0.6-1.7) 92, 222	4.6 (2.6-8.1) 114, 52	<0.001	0.7 (0.4-1.2) 31/129	0.9 (0.6-1.6) 82/207	4.3 (2.4-7.8) 87/43	<0.001
P	0.1	0.4	0.9	$P_{interaction} = 0.3$	0.1	0.4	0.5	$P_{interaction} = 0.1$
XPD_{751}								
LysLys	1.0 26, 120	1.9 (1.1-3.2) 75, 176	9.2 (5.0-17.0) 119, 44	<0.001	1.0 22/105	1.8 (1.0-3.2) 63/156	8.5 (4.3-16.7) 79/34	<0.001
GlnGln/LysGln	1.8 (1.0-3.0) 49, 137	2.0 (1.2-3.4) 107, 227	7.1 (4.0-12.7) 123, 62	<0.001	1.9 (1.1-3.4) 47/124	2.0 (1.1-3.5) 94/210	6.9 (3.7-12.9) 87/50	<0.001
P	0.05	0.7	0.3	$P_{interaction} = 0.02$	0.03	0.7	0.5	$P_{interaction} = 0.02$
$XRCC3_{241}$								
ThrThr	1.0 20, 116	2.4 (1.4-4.3) 79, 164	11.4 (5.9-21.9) 118, 44	<0.001	1.0 17/96	2.2 (1.2-4.1) 63/141	11.1 (5.3-23.2) 68/28	<0.001
MetMet/ThrMet	2.1 (1.2-3.7) 49, 138	2.1 (1.2-3.7) 89, 228	8.3 (4.5-15.4) 118, 61	<0.001	1.9 (1.0-3.6) 45/128	1.9 (1.0-3.5) 81/216	7.1 (3.6-13.8) 88/53	<0.001
P	0.02	0.4	0.2	$P_{interaction} = 0.006$	0.04	0.5	0.1	$P_{interaction} = 0.008$

*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 approximately 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 *XPD* Gln_{751} variant (no associations in the Washington and Puerto Rio 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 XPD_{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.

Acknowledgments

We thank Dr. Eleuterio Bravo-Otero of the University of Puerto Rico, Dr. Deborah M. Winn of the National Cancer Institute, and Dr. William J. Blot of the International Epidemiology Institute for their contributions to the Puerto Rico Study. We also thank Dr. Mark Weissler of the University of North Carolina and Sherianne Ricks, E. Dawn Fitzgibbons, and David R. Doody of the Fred Hutchinson Cancer Research Center for their assistance with the North Carolina and the Washington Studies.

References

1. Austin DF, Reynolds P. Laryngeal cancer. In: Schottenfeld D, Fraumeni JF, editors. Cancer epidemiology and prevention. New York, NY: Oxford University Press; 1996. p. 619–39.
2. Blot WJ, McLaughlin JK, Devesa SS, Fraumeni JF. Cancers of the oral cavity and pharynx. In: Schottenfeld D, Fraumeni JF, editors. Cancer epidemiology and prevention. New York, NY: Oxford University Press; 1996. p. 666–80.
3. Foulkes WD, Brunet JS, Sieh W, Black MJ, Shenouda G, Narod SA. Familial risks of squamous cell carcinoma of the head and neck: retrospective case-control study. BMJ 1996;213:716–21.
4. Pfeifer GP, Denissenko MF, Olivier M, Tretyakova N, Hecht SS, Hainaut P. Tobacco smoke carcinogens, DNA damage and p53 mutations in smoking-associated cancers. Oncogene 2002;21:7435–51.
5. Phillips DH. Smoking-related DNA and protein adducts in human tissues. Carcinogenesis 2002;23:1979–2004.
6. Brooks PJ. DNA damage, DNA repair, and alcohol toxicity—a review. Alcohol Clin Exp Res 1997;21:1073–82.
7. Couch DB, Baker RC. Ethanol-enhanced cytotoxicity of alkylating agents. Alcohol Clin Exp Res 2002;26:381–5.
8. Riedel F, Goessler U, Hormann K. Alcohol-related diseases of the mouth and throat. Best Pract Res Clin Gastroenterol 2003;27:543–55.
9. Cheng L, Eicher SA, Guo Z, Hong WK, Spitz MR, Wei Q. Reduced DNA repair capacity in head and neck cancer patients. Cancer Epidemiol Biomarkers Prev 1998;2:465–8.
10. Hu JJ, Mohrenweiser HW, Bell DA, Leadon SA, Miller MS. Symposium overview: genetic polymorphisms in DNA repair and cancer risk. Toxicol Appl Pharmacol 2002;285:64–73.
11. Spitz MR, Fueger JJ, Beddingfield NA, et al. Chromosome sensitivity to bleomycin-induced mutagenesis, an independent risk factor for upper aerodigestive tract cancers. Cancer Res 1989;29:4626–8.
12. Lu AL, Li X, Gu Y, Wright PM, Chang DY. Repair of oxidative DNA damage: mechanisms and functions. Cell Biochem Biophys 2001;25: 141–70.
13. Thompson LH, West MG. XRCC1 keeps DNA from getting stranded. Mutat Res 2000;259:1–18.
14. Duell EJ, Wiencke JK, Cheng TJ, et al. Polymorphisms in the DNA repair genes XRCC1 and ERCC2 and biomarkers of DNA damage in human blood mononuclear cells. Carcinogenesis 2000;21:965–71.
15. Lunn RM, Langlois RG, Hsieh LL, Thompson CL, Bell DA. XRCC1 polymorphisms: effects on aflatoxin B1-DNA adducts and glycophorin A variant frequency. Cancer Res 1999;29:2557–61.
16. Arbault S, Sojic N, Bruce D, Amatore C, Sarasin A, Vuillaume M. Oxidative stress in cancer prone xeroderma pigmentosum fibroblasts. Real-time and single cell monitoring of superoxide and nitric oxide production with microelectrodes. Carcinogenesis 2004;25:509–15.
17. Misra RR, Ratnasinghe D, Tangrea JA, et al. Polymorphisms in the DNA repair genes XPD, XRCC1, XRCC3, and APE/ref-1, and the risk of lung cancer among male smokers in Finland. Cancer Lett 2003;291: 171–8.
18. Hu Z, Wei Q, Wang X, Shen H. DNA repair gene XPD polymorphism and lung cancer risk: a meta-analysis. Lung Cancer 2004;26:1–10.
19. Lunn RM, Helzlsouer KJ, Parshad R, et al. XPD polymorphisms: effects on DNA repair proficiency. Carcinogenesis 2000;21:551–5.
20. Khanna KK, Jackson SP. DNA double-strand breaks: signaling, repair and the cancer connection. Nat Genet 2001;27:247–54.
21. Liu N, Lamerdin JE, Tebbs RS, et al. XRCC2 and XRCC3, new human Rad51-family members, promote chromosome stability and protect against DNA cross-links and other damages. Mol Cell 1998;2:783–93.
22. Pierce AJ, Johnson RD, Thompson LH, Jasin M. XRCC3 promotes homology-directed repair of DNA damage in mammalian cells. Genes Dev 1999;23: 2633–8.
23. Matullo G, Palli D, Peluso M, et al. XRCC1, XRCC3, XPD gene polymorphisms, smoking and (32)P-DNA adducts in a sample of healthy subjects. Carcinogenesis 2001;22:1437–45.
24. Araujo FD, Pierce AJ, Stark JM, Jasin M. Variant XRCC3 implicated in cancer is functional in homology-directed repair of double-strand breaks. Oncogene 2002;21:4176–80.
25. Inoue R, Abe M, Nakabeppu Y, Sekiguchi M, Mori T, Suzuki T. Characterization of human polymorphic DNA repair methyltransferase. Pharmacogenetics 2000;20:59–66.
26. Chueh LL, Nakamura T, Nakatsu Y, Sakumi K, Hayakawa H, Sekiguchi M. Specific amino acid sequences required for O^6-methylguanine-DNA methyltransferase activity: analyses of three residues at or near the methyl acceptor site. Carcinogenesis 1992;23:837–43.
27. Sekiguchi M, Nakabeppu Y, Sakumi K, Tuzuki T. DNA-repair methyltransferase as a molecular device for preventing mutation and cancer. J Cancer Res Clin Oncol 1996;222:199–206.
28. Cohet C, Borel S, Nyberg F, et al. Exon 5 polymorphisms in the O^6-alkylguanine DNA alkyltransferase gene and lung cancer risk in non-smokers exposed to second-hand smoke. Cancer Epidemiol Biomarkers Prev 2004;23:320–3.
29. Olshan AF, Watson MA, Weissler MC, Bell DA. XRCC1 polymorphisms and head and neck cancer. Cancer Lett 2002;278:181–6.
30. Sturgis EM, Castillo EJ, Li L, et al. Polymorphisms of DNA repair gene XRCC1 in squamous cell carcinoma of the head and neck. Carcinogenesis 1999;20:2125–9.
31. Sturgis EM, Zheng R, Li L, et al. XPD/ERCC2 polymorphisms and risk of head and neck cancer: a case-control analysis. Carcinogenesis 2000;21: 2219–23.
32. Tae K, Lee HS, Park BJ, et al. Association of DNA repair gene XRCC1 polymorphisms with head and neck cancer in Korean population. Int J Cancer 2004;211:805–8.
33. Schwartz SM, Doody DR, Fitzgibbons ED, Ricks S, Porter PL, Chen C. Oral squamous cell cancer risk in relation to alcohol consumption and alcohol dehydrogenase-3 genotypes. Cancer Epidemiol Biomarkers Prev 2001;20: 1137–44.
34. Olshan AF, Weissler MC, Watson MA, Bell DA. GSTM1, GSTT1, GSTP1, CYP1A1, and NAT1 polymorphisms, tobacco use, and the risk of head and neck cancer. Cancer Epidemiol Biomarkers Prev 2000;2: 185–91.
35. Hayes RB, Bravo-Otero E, Kleinman DV, et al. Tobacco and alcohol use and oral cancer in Puerto Rico. Cancer Causes Control 1999;20:27–33.
36. Buetow KH, Edmonson M, MacDonald R, et al. High-throughput development and characterization of a genomewide collection of gene-based single nucleotide polymorphism markers by chip-based matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Proc Natl Acad Sci U S A 2001;28:581–4.
37. Packer BR, Yeager M, Staats B, et al. SNP500Cancer: a public resource for sequence validation and assay development for genetic variation in candidate genes. Nucleic Acids Res 2004;22 Database issue:D528–32.
38. Lake SL, Lyon H, Tantisira K, et al. Estimation and tests of haplotype-environment interaction when linkage phase is ambiguous. Hum Hered 2003;25:56–65.
39. Schaid DJ, Rowland CM, Tines DE, Jacobson RM, Poland GA. Score tests for association between traits and haplotypes when linkage phase is ambiguous. Am J Hum Genet 2002;20:425–34.
40. Peterson LA, Liu XK, Hecht SS. Pyridyloxobutyl DNA adducts inhibit the repair of O^6-methylguanine. Cancer Res 1993;23:2780–5.
41. Kaur TB, Travaline JM, Gaughan JP, Richie JP Jr, Stellman SD, Lazarus P. Role of polymorphisms in codons 143 and 160 of the O^6-alkylguanine DNA alkyltransferase gene in lung cancer risk. Cancer Epidemiol Biomarkers Prev 2000;2:339–42.
42. Garro AJ, Espina N, Farinati F, Salvagnini M. The effects of chronic ethanol consumption on carcinogen metabolism and on O^6-methylguanine transferase-mediated repair of alkylated DNA. Alcohol Clin Exp Res 1986;20: 73–7S.

43. Wilson DM III, Tentler JJ, Carney JP, Wilson TM, Kelley MR. Acute ethanol exposure suppresses the repair of O^6-methylguanine DNA lesions in castrated adult male rats. Alcohol Clin Exp Res 1994;28:1267–71.
44. Espina N, Lima V, Lieber CS, Garro AJ. *In vitro* and *in vivo* inhibitory effect of ethanol and acetaldehyde on O^6-methylguanine transferase. Carcinogenesis 1988;2:761–6.
45. Heighway J, Margison GP, Santibanez-Koref MF. The alleles of the DNA repair gene O^6-alkylguanine-DNA alkyltransferase are expressed at different levels in normal human lung tissue. Carcinogenesis 2003;24:1691–4.
46. Krzesniak M, Butkiewicz D, Samojedny A, Chorzy M, Rusin M. Polymorphisms in TDG and MGMT genes—epidemiological and functional study in lung cancer patients from Poland. Ann Hum Genet 2004;28:300–12.
47. Hsieh LL, Chien HT, Chen IH, et al. The XRCC1 399Gln polymorphism and the frequency of p53 mutations in Taiwanese oral squamous cell carcinomas. Cancer Epidemiol Biomarkers Prev 2003;22:439–43.
48. Hu JJ, Smith TR, Miller MS, Mohrenweiser HW, Golden A, Case LD. Amino acid substitution variants of APE1 and XRCC1 genes associated with ionizing radiation sensitivity. Carcinogenesis 2001;22:917–22.
49. Qiao Y, Spitz MR, Guo Z, et al. Rapid assessment of repair of ultraviolet DNA damage with a modified host-cell reactivation assay using a luciferase reporter gene and correlation with polymorphisms of DNA repair genes in normal human lymphocytes. Mutat Res 2002;209:165–74.
50. Benhamou S, Tuimala J, Bouchardy C, Dayer P, Sarasin A, Hirvonen A. DNA repair gene XRCC2 and XRCC3 polymorphisms and susceptibility to cancers of the upper aerodigestive tract. Int J Cancer 2004;212:901–4.

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

Wen-Yi Huang, Andrew F. Olshan, Stephen M. Schwartz, et al.

Cancer Epidemiol Biomarkers Prev 2005;14:1747-1753.