Null Results in Brief

No Association between *hOGG1* Ser^{326}Cys Polymorphism and Risk of Squamous Cell Carcinoma of the Head and Neck

Zhengdong Zhang,[1] Qiuling Shi,[1] Li-E Wang,[1] Erich M. Sturgis,[1,2] Margaret R. Spitz,[1] Adel K. El-Naggar,[3] Waun K. Hong,[4] and Qingyi Wei[1]

Departments of [1]Epidemiology, [2]Head and Neck Surgery, [3]Pathology, and [4]Thoracic and Head and Neck Medical Oncology, University of Texas M.D. Anderson Cancer Center, Houston, Texas

Introduction

Squamous cell carcinoma of the head and neck (SCCHN) is one of the most common cancers in the world (1). In the United States, it is estimated that there was 37,200 newly diagnosed SCCHN cases and 11,000 deaths in 2003 (2). Tobacco smoke and alcohol use are the major risk factors for SCCHN; however, only a fraction of individuals exposed to tobacco smoke or alcohol develop SCCHN, suggesting that there is variation in individual susceptibility to the disease. Therefore, SCCHN is an excellent disease model for the study of gene-environment interaction. One of the genotoxic effects of tobacco is oxidative DNA damage induced by reactive oxygen species, and 8-hydroxy-2-deoxyguanosine is one of the most common forms of oxidative DNA damage and is a marker of cellular oxidative stress (3). hOGG1 catalyzes the removal of 8-hydroxy-2-deoxyguanosine and cleavage of DNA at the AP site as part of the base excision repair pathway (4). At least 10 polymorphisms of *hOGG1* have been identified (5), one of which is a C → G at bp 1245 (C1245G) in the 1α-specific exon 7 that causes an amino acid substitution from serine to cysteine in codon 326 (Ser^{326}Cys), potentially resulting in functional alteration (6). Several association studies of this *hOGG1* C1245G polymorphism and cancer risk have generated contradicting results (3). Two recent larger breast cancer case-control studies yielded negative results (7, 8), but one recent study on SCCHN was positive (9). To further verify the possible role of the *hOGG1* C1245G polymorphism in the etiology of SCCHN, we investigated the association between this polymorphism and the risk of SCCHN in a large hospital-based case-control study of 706 patients with SCCHN and 1,196 cancer-free control subjects.

Cancer Epidemiol Biomarkers Prev 2004;13(6):1081–3

Received 12/1/03; accepted 2/2/04.

Grant support: NIH grants CA 86390 and CA 97007 (M.R. Spitz and W.K. Hong), ES 11740 (Q. Wei), and CA 16672 (M. D. Anderson Cancer Center).

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: Qingyi Wei, Department of Epidemiology, Unit 189, University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030. Phone: 713-792-3020; Fax: 713-563-0999. E-mail: qwei@mdanderson.org

Materials and Methods

Study Population. All patients at the University of Texas M. D. Anderson Cancer Center who were newly diagnosed with histologically confirmed SCCHN between May 1, 1995 and September 30, 2003 were eligible for this study. The cancer-free control subjects were genetically unrelated to the patients with SCCHN and were recruited from among two populations: they included 558 enrollees in a multispecialty physician practice, the Kelsey Seybold Foundation, which has multiple clinics throughout the Houston metropolitan area (10), and 638 M. D. Anderson Cancer Center visitors accompanying patients to our cancer center outpatient clinics. To check for any selection bias in terms of genotypes, these two control populations were first compared for the distribution of the *hOGG1* CC, CG, and GG genotypes, and no difference was found (χ^2 = 3.863; P = 0.145). Therefore, these controls were combined in the final analysis to increase the study power. These control subjects were frequency matched to the case subjects on age (±5 years), sex, and smoking status (never, ever, and current). Informed consent was obtained, and all subjects agreed to donate 30 mL of blood for biomarker testing and complete a detailed questionnaire eliciting their demographic, exposure to tobacco smoke and alcohol, and family history information. The study was approved by both our institutional review board and the Kelsey Seybold Foundation review board.

Genotyping. We used the published primer sequences and PCR-RFLP method (11) to amplify a 200-bp fragment of genomic DNA. The restriction enzyme *Fnu*4HI (New England Biolabs, Inc., Beverly, MA) was used to type the *hOGG1* C1245G polymorphism. The variant allele was cut by the enzyme into two 100-bp fragments. The fragments were separated on a 3% agarose gel (Fig. 1). More than 10% of the samples were randomly selected for repeat assays, and the results were 100% concordant.

Statistical Analysis. We calculated crude and adjusted odds ratios (OR) and their 95% confidence intervals (95% CI) for the *hOGG1* C1245G genotypes by using univariate and multivariate logistic regression analyses.

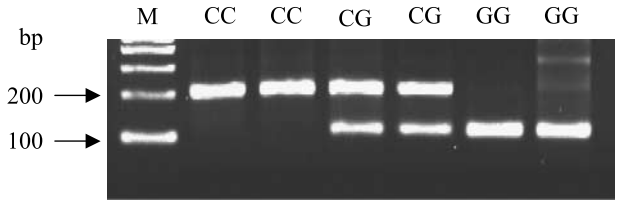

Figure 1. PCR-RFLP analysis for the *hOGG1* C1245G polymorphism at codon 326 (Ser^{326}Cys) in exon 7. The 200-bp PCR product was digested by *Fnu*4HI into two 100-bp fragments if it was the G variant allele or undigested if it was the C allele.

All statistical analyses were performed with the Statistical Analysis System software (version 8.2, SAS Institute, Inc., Cary, NC).

Results

The 706 cases (75.5% males) with SCCHN included oral cavity [209 (29.6%)], pharynx [325 (46.0%)], and larynx [172 (24.4%)], and the 1,196 cancer-free control subjects (73.2% males) were non-Hispanic whites; there was no significant difference in mean (± SD) age between the cases (56.9 ± 11.8) and the controls (56.7 ± 11.1). The *hOGG1* genotype and allele frequencies were not significantly different between cases and controls (Table 1), and the genotypes in the controls were in Hardy-Weinberg equilibrium (P = 0.06). The frequencies of the *hOGG1* variant GG homozygous genotype and the G allele in the cases were not different from those in the controls (5.5% vs. 5.8%; P = 0.802, respectively; 0.211 vs. 0.220; P = 0.687, respectively).

On logistic regression analysis, neither the homozygous GG (adjusted OR 0.98, 95% CI 0.65-1.48) nor the heterozygous CG (adjusted OR 0.93, 95% CI 0.76-1.14) genotypes was associated with SCCHN risk after adjustment for age, sex, and smoking and alcohol status. No significant findings were evident in further stratified analysis, and there was no evidence of any interaction between the *hOGG1* genotype and other covariates (data not shown).

Statistical Power. We had 80% power (two-sided test, α = 0.05) to detect an OR of 1.66 for GG homozygotes (5.8% in the controls), if this variant genotype is a risk genotype, compared with the CC+CG genotype. For both variant genotypes (CG + GG; 38.2% in the controls), the detectable OR was 1.31 compared with the CC genotype. Thus, our study had sufficient power to detect the ORs reported by Elahi et al. (9); i.e., OR 4.1, 95% CI 1.3-13.0 for GG homozygotes and OR 1.6, 95% CI 1.04-2.6 for variant genotypes (CG + GG).

Discussion

In the present study, we investigated the association between the *hOGG1* C1245G (Ser^{326}Cys) polymorphism and SCCHN risk, but we did not find evidence to support such an association. Our findings suggest that this common polymorphism may not play a major role in the etiology of SCCHN. To the best of our knowledge, this is the largest molecular epidemiologic study of this kind ever reported to date.

One reason for discrepant results in case-control studies is that the selection of the controls may be biased in terms of biomarkers of interest. The frequencies of the *hOGG1* variant GG homozygous genotype (5.8%) and the G allele (0.220) in our study are consistent with those of 434 female white control subjects (4.6% and 0.241, respectively) in a recent Danish population-based nested breast cancer case-control study (7) but are higher than those of 338 white control subjects (1.8% and 0.130, respectively) in an earlier positive U.S. hospital-based orolaryngeal cancer case-control study (9). It is interesting that our cases and the cases of these two studies exhibited similar frequencies of GG homozygous genotype (5.5%, 5.2%, and 5.4%, respectively) and the G allele (0.211, 0.224, and 0.220). Other published case-control studies had much smaller numbers (<300 for each ethnic group) of controls with the frequency of the GG homozygous genotype ranging from ~2% to 9% for white and between 13% and 24% for Asians (3, 12). The reported lowest GG homozygous genotype in control subjects was 1.8% in white (9) and the reported highest was 24.2% in Chinese (12).

Early association studies of Japanese populations suggested a possible role of the common variant of *hOGG1* 1245G in susceptibility to lung cancer, but the results of the most recent Japanese study of lung adenocarcinoma, the most plausible oxidative damage-induced cell type, did not support such an association (13). In the only published U.S. case-control study of 169

Table 1. SCCHN Risk Associated with *hOGG1* Ser^{326}Cys Polymorphism

Genotypes	Cases (n = 706)	Controls (n = 1,196)*	Crude OR (95% CI)	Adjusted OR (95% CI)[c]
	n (%)	n (%)		
hOGG1 C1245G				
CC	447 (63.3)	739 (61.8)	1.00 (reference)	1.00 (reference)
CG	220 (31.2)	388 (32.4)	0.94 (0.77-1.15)	0.93 (0.76-1.14)
GG	39 (5.5)	69 (5.8)	0.93 (0.62-1.41)	0.98 (0.65-1.48)
CG/GG	259 (36.7)	457 (38.2)	0.94 (0.77-1.14)	0.94 (0.77-1.14)
Number of allele	1,412	2,392		
G allele[b]	0.211	0.220		

*The observed genotype frequency in the control subjects was in agreement with Hardy-Weinberg equilibrium ($p^2 + 2pq + q^2 = 1$; $\chi^2 = 3.54$; $P = 0.06$), and no difference in the distribution of the genotypes was evident ($\chi^2 = 0.440$; $P = 0.802$).
[c]ORs were adjusted for age, sex, and smoking and alcohol status in a logistic regression model.
[b]$\chi^2 = 0.403$; $P = 0.687$ from χ^2 test for the G allele frequencies between cases and controls.

white patients with orolaryngeal cancer and 338 control subjects, Elahi et al. (9) found a significant association between the *hOGG1* 1245CG or 1245GG variant genotype and risk of orolaryngeal cancer, particularly in smokers and alcohol drinkers. Nevertheless, the positive finding of that study is weakened by the small sample size and the fact that the frequencies of variant alleles and genotypes were the lowest in the controls of that study among published studies and our study.

In conclusion, we did not find evidence to support an association between *hOGG1* C1245G polymorphism and SCCHN risk in our study population. The limitation of our study is the hospital-based study design, and we cannot rule out the possibility of selection bias of subjects. Given that many genes are involved in the repair of oxidative damage (3), the role of oxidative damage and repair in SCCHN may be more efficiently investigated by using a phenotypic assay (14) and performing genotype and phenotype correlation analysis and by comprehensive study of the entire repair pathway (15).

Acknowledgments

We thank Margaret Lung and Peggy Schuber for their assistance in recruiting the subjects; Jianzhong He, John I. Calderon, and Kejin Xu for their laboratory assistance; Ann Sutton for scientific editing; and Joanne Sider for manuscript preparation.

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