Null Results in Brief

CYP2E1 G1532C, *NQO1* Pro187Ser, and *CYP1B1* Val432Leu Polymorphisms Are Not Associated with Risk of Squamous Cell Carcinoma of the Head and Neck

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Introduction

Tobacco use and alcohol consumption are the major risk factors for squamous cell carcinoma of the head and neck (SCCHN; ref. 1). Polymorphisms in tobacco carcinogen– and alcohol-metabolizing genes may contribute to variation in enzyme activities and, thus, influence individual susceptibility to SCCHN.

Cytochrome P450 2E1 (CYP2E1) is a phase I enzyme that catalyzes metabolic activation of several compounds found in cigarette smoke, such as *N*-nitroso-dimethylamine, benzene, *N*-nitrosonornicotine (2), and ethanol (3). More than 25 polymorphisms of *CYP2E1* have been identified,[3] one of which is G1532C located upstream of the *CYP2E1* transcription start site that is believed to affect CYP2E1 expression (3).

Cytochrome P450 1B1 (CYP1B1) is another phase I enzyme that activates tobacco-related carcinogens, such as polycyclic aromatic hydrocarbons and aromatic amines (4). Among the eight potential functional polymorphisms,[3] a G-to-A substitution resulting in an amino acid change from valine to leucine at codon 432 (V432L) may alter catalytic efficiency for the 4-hydroxylation (5).

NAD(P)H:quinone oxidoreductase 1 (NQO1), a cytosolic two-electron reductase (a phase II enzyme), catalyzes reductive activation of carcinogens from cigarette smoke, such as nitrosamines and heterocyclic amines (6), and protects cells against oxidative damage from reactive oxygen species (7). Among 14 documented polymorphisms, one functional polymorphism, a C-to-T substitution at nucleotide 609 of exon 6, causes an amino acid change from proline to serine at codon 187 (P187S; ref. 8), resulting in a loss of NQO1 activity (9).

Previously published association studies have led to examination of *CYP2E1*, *NQO1*, and *CYP1B1* polymorphisms in relation to the risk of some tobacco-related carcinomas but only few relatively small studies have examined SCCHN risk. To further verify the possible roles of these polymorphisms in the etiology of SCCHN, we investigated the associations between these three functional polymorphisms and the risk of SCCHN in a large hospital-based, case-control study in a non-Hispanic White population.

Materials and Methods

Study Population. Cases were patients with newly diagnosed, histologically confirmed, previously untreated SCCHN seen at the University of Texas M. D. Anderson Cancer Center between May 1995 and October 2003 (10). Cancer-free controls were hospital visitors who were genetically unrelated to the SCCHN patients and were frequency-matched with cases for age (±5 years), sex, and smoking status (current, former, and never), and ethnicity. After informed consent was obtained, a blood specimen was obtained from each study subject.

Control subjects were from among two populations: 602 enrollees in the Kelsey Seybold Foundation, a multispecialty physician practice with multiple clinics throughout the Houston metropolitan area (11), and 624 visitors to M. D. Anderson Cancer Center who were accompanying patients to our outpatient clinics. To minimize selection bias in either control group in terms of genotypes and unknown confounders and to increase the study power, we combined these subgroups into a single control group in the final analysis after we had found no differences in the frequency distributions of the *CYP2E1* G1532C, *NQO1* Pro187Ser, and *CYP1B1* Val432Leu genotypes between the two subgroups ($\chi^2 = 3.18$, $P = 0.204$; $\chi^2 = 0.103$, $P = 0.950$; and $\chi^2 = 1.99$, $P = 0.370$; respectively).

Genotyping. DNA was extracted from the buffy coat of whole blood with the Qiagen DNA Blood Mini kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. Genotyping was done by using published primer sequences and PCR-RFLP methods for *CYP2E1* G1532C, *NQO1* Pro187Ser, and *CYP1B1* Val432Leu polymorphisms (12-14). More than 10% of the samples were randomly selected for repeat genotyping and the results were in 100% concordance.

Statistical Analysis. We assessed the associations between genetic polymorphisms and risk of SCCHN after controlling for age, sex, and other potential confounding factors by computing odds ratios (OR) and 95% confidence intervals (95% CI) from unconditional logistic regression analyses using SAS software (SAS version 8.2, SAS Institute, Inc., Cary, NC).

Results

Cases were 724 non-Hispanic Whites (74.7% male) with SCCHN of the oral cavity (224, 30.9%), pharynx (325, 44.9%), or larynx (175, 24.2%); controls were 1,226 non-Hispanic cancer-free Whites (73.9% male). Mean (±SD) age was not

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[3] http://snp500cancer.nci.nih.gov/snplist.cfm.

Table 1. SCCHN risk associated with *CYP1B1* V432L, *CYP2E1* G1532C, and *NQO1* P187S polymorphisms

Genotype	Case patients (n = 724)	Control subjects (n = 1,226)	Crude OR (95% CI)	Adjusted OR (95% CI)*
	No. (%)	No. (%)		
CYP1B1 V432L†				
Val/Val	238 (32.9)	365 (29.8)	1.00	1.00
Val/Leu	342 (47.2)	605 (49.3)	0.87 (0.70-1.07)	0.86 (0.70-1.07)
Leu/Leu	144 (19.9)	256 (20.9)	0.86 (0.66-1.12)	0.89 (0.68-1.16)
Val/Leu + Leu/Leu	486 (67.1)	861 (70.2)	0.87 (0.71-1.06)	0.87 (0.72-1.06)
Leu allele	0.435	0.456		
CYP2E1 G1532C†				
GG	684 (94.5)	1137 (92.7)	1.00	1.00
CG	37 (5.1)	86 (7.03)	0.72 (0.48-1.06)	0.73 (0.49-1.10)
CC	3 (0.4)	3 (0.23)	1.66 (0.34-8.26)	1.97 (0.39-9.86)
CG + CC	40 (5.5)	89 (7.23)	0.75 (0.51-1.10)	0.77 (0.52-1.13)
C allele	0.030	0.038		
NQO1 Pro187Ser†				
Pro/Pro	484 (66.8)	805 (65.7)	1.00	1.00
Ser/Pro	209 (28.9)	388 (31.6)	0.89 (0.73-1.10)	0.89 (0.73-1.09)
Ser/Ser	31 (4.3)	33 (2.7)	1.56 (0.95-2.58)	1.56 (0.94-2.59)
Ser/Pro + Ser/Ser	240 (33.2)	421 (34.3)	0.95 (0.78-1.15)	0.94 (0.78-1.15)
Ser allele	0.187	0.185		

*Adjusted for age, sex, smoking status, and alcohol use in a logistic regression model.
†Genotype distributions: $\chi^2 = 3.19$, $P = 0.203$, *CYP2E1*; $\chi^2 = 4.75$, $P = 0.093$, *NQO1*; and $\chi^2 = 2.05$, $P = 0.359$, *CYP1B1*, respectively. Allele frequency: $\chi^2 = 1.83$, $P = 0.176$, *CYP2E1*; $\chi^2 = 0.02$, $P = 0.877$, *NQO1*; and $\chi^2 = 1.60$, $P = 0.205$, *CYP1B*, respectively. The observed genotype frequency in the control subjects was in agreement with Hardy-Weinberg equilibrium ($p^2 + 2pq + q^2 = 1$): $\chi^2 = 1.02$, $P = 0.314$, *CYP2E1*; $\chi^2 = 2.92$, $P = 0.087$, *NQO1*; and $\chi^2 = 0.03$, $P = 0.856$, *CYP1B*, respectively.

different between the cases (57.1 ± 11.9) and the controls (57.1 ± 11.6; $P = 0.983$). Frequency distributions of *CYP2E1*, *NQO1*, and *CYP1B1* genotypes and their associations with SCCHN risk are shown in Table 1. Distributions of these genotypes and allele frequencies were not significantly different between cases and controls, and the genotypes in the controls were in Hardy-Weinberg equilibrium ($P = 0.314$, *CYP2E1*; $P = 0.087$, *NQO1*; and $P = 0.359$, *CYP1B1*; respectively). No significant associations between genetic polymorphisms of *CYP2E1*, *NQO1*, and *CYP1B1* and risk of SCCHN were observed after adjustment for age, sex, and smoking and alcohol status (Table 1). We then stratified the data by cancer sites, smoking and alcohol status, age, and sex, but did not find any evidence of associations between the genotypes and SCCHN risk among these subgroups (data not shown). Furthermore, we found no evidence of any interaction between *CYP2E1*, *NQO1*, and *CYP1B1* genotypes and other covariates (data not shown).

For this analysis, we had an 80% power (two-sided test, $\alpha = 0.05$) to detect an OR of 0.71 or 1.41 for *CYP1B1* Leu/Leu homozygotes (20.9% in the controls) if the variant genotype was a protective or risk genotype compared with the *CYP1B1* Val/Val + Val/Leu genotype, assuming a recessive allele model. Similarly, for *CYP1B1* variant genotypes (Val/Val + Val/Leu, 70.2% in the controls), the detectable OR was 0.76 or 1.31 compared with the Val/Val genotype, assuming a dominant allele model. For the combined *CYP2E1* CG + CC (7.35% in the controls) and *NQO1* Ser/Pro + Ser/Ser (34.3% in the controls) genotypes, we had an 80% power (two-sided test, $\alpha = 0.05$) to detect an OR of 0.56 or 1.79 and 0.75 or 1.33 compared with the *CYP2E1* GG and *NQO1* Pro/Pro genotypes, respectively. Thus, our study had sufficient power to detect an OR of 0.78 ($P > 0.05$ for *CYP1B1* Leu/Leu versus Val/Val genotype) reported in a study of 312 SCCHN cases and 300 controls by Ko et al. (15) and an OR of 0.87 ($P > 0.05$ for *CYP2E1* CG + CC versus GG genotype) reported in a study of 75 SCCHN cases and 200 controls by Gonzalez et al. (12).

Discussion

Cancers of the lung, bladder, and head and neck are all tobacco related. Previous studies suggested that functional polymorphisms of *CYP1B1*, *CYP2E1*, and *NQO1* were strongly associated with risk of lung and bladder carcinomas (16-19), but few studies have explored these polymorphisms as risk factors for SCCHN. In the present study, among non-Hispanic Whites, we found no evidence of an association between the *CYP2E1* G1532C, *NQO1* Pro187Ser, and *CYP1B1* Val432Leu polymorphisms and SCCHN risk. Our findings suggest that these polymorphisms may not play a major role in the etiology of SCCHN.

To the best of our knowledge, ours is the largest molecular epidemiologic study of the association between these polymorphisms and SCCHN risk. Only two previous studies examined the *CYP1B1* Val432Leu and *CYP2E1* G1532C polymorphisms and the risk of SCCHN. Ko et al. (15) found that *CYP1B1* Val432Leu polymorphism had no main effect (312 cases and 300 controls) but was a susceptibility factor among smokers, which was not confirmed by our study. The frequency of the *CYP1B1* genotypes among our larger control group were 29.8% for the Val/Val genotype, 49.3% for Val/Leu genotype, and 20.9 for Leu/Leu genotype, compared with 36.3%, 46.0%, and 17.7% in the study by Ko et al. (15). The discrepancy in distribution may reflect differences in ethnic backgrounds of the two studies (European versus American Caucasians) or a chance finding in the study by Ko et al. in smokers of 195 cases and 177 controls compared with 536 cases and 857 controls who were smokers in our study. Another previous case-control study of 75 SCCHN cases and 200 controls showed that the *CYP2E1* G1532C polymorphism was not associated with SCCHN risk (12), a finding we confirmed in this larger study. This finding is likely to be true because control subjects of both studies had similar frequencies of *CYP2E1* G1532C polymorphism genotypes. To date, no reports have been published as to whether the *NQO1* Pro187Ser polymorphism is associated with SCCHN risk. Our data did not support the hypothesis that this polymorphism had an effect on SCCHN risk, although one study found that individuals carrying 1 or 2 *NQO1* 609T alleles had a 2.85-fold higher risk for the development of esophageal adenocarcinoma ($n = 61$ versus 252 controls) and a 2.18-fold higher risk for cardiac adenocarcinoma ($n = 120$ versus 252 controls), respectively, than wild-type homozygotes (20). However, these positive findings may have been due to chance because of the small sample sizes, particularly in the subgroups; moreover, that study had higher Pro/Pro (73.4%)

and lower Ser/Pro (25.0%) genotype frequencies among controls compared with 65.7% for Pro/Pro and 31.6% for Ser/Pro in our study.

The strengths of our study include the relatively large sample size of a single ethnic group for a relatively rare cancer and the study power to detect a reasonably small risk. The limitation of our study was the hospital-based study design with which we could not exclude the possibility of selection bias that may have masked a real association. Because the *CYP2E1*, *NQO1*, and *CYP1B1* genes all have multiple putative functional polymorphisms, large population-based case-control studies with genotyping of more variants and subsequent haplotype analysis are warranted to understand the roles of these polymorphisms in the etiology of SCCHN.

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