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Research Article

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Joint Effects of Alcohol Consumption and Polymorphisms in Alcohol and Oxidative Stress Metabolism Genes on Risk of Head and Neck Cancer

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Abstract

Background: Single-nucleotide polymorphisms (SNP) in alcohol metabolism genes are associated with squamous cell carcinoma of the head and neck (SCCHN) and may influence cancer risk in conjunction with alcohol. Genetic variation in the oxidative stress pathway may impact the carcinogenic effect of reactive oxygen species produced by ethanol metabolism. We hypothesized that alcohol interacts with these pathways to affect SCCHN incidence.

Methods: Interview and genotyping data for 64 SNPs were obtained from 2,552 European- and African-American subjects (1,227 cases and 1,325 controls) from the Carolina Head and Neck Cancer Epidemiology Study, a population-based case–control study of SCCHN conducted in North Carolina from 2002 to 2006. We estimated ORs and 95% confidence intervals (CI) for SNPs and haplotypes, adjusting for age, sex, race, and duration of cigarette smoking. *P* values were adjusted for multiple testing using Bonferroni correction.

Results: Two SNPs were associated with SCCHN risk: *ADH1B* rs1229984 A allele (OR = 0.7; 95% CI, 0.6–0.9) and *ALDH2* rs2238151 C allele (OR = 1.2; 95% CI, 1.1–1.4). Three were associated with subsite tumors: *ADH1B* rs17028834 C allele (larynx, OR = 1.5; 95% CI, 1.1–2.0), *SOD2* rs4342445 A allele (oral cavity, OR = 1.3; 95% CI, 1.1–1.6), and *SOD2* rs5746134 T allele (hypopharynx, OR = 2.1; 95% CI, 1.2–3.7). Four SNPs in alcohol metabolism genes interacted additively with alcohol consumption: *ALDH2* rs2238151, *ADH1B* rs1159918, *ADH7* rs1154460, and *CYP2E1* rs2249695. No alcohol interactions were found for oxidative stress SNPs.

Conclusions and Impact: Previously unreported associations of SNPs in *ALDH2*, *CYP2E1*, *GPX2*, *SOD1*, and *SOD2* with SCCHN and subsite tumors provide evidence that alterations in alcohol and oxidative stress pathways influence SCCHN carcinogenesis and warrant further investigation. *Cancer Epidemiol Biomarkers Prev; 20(11); 2438–49. ©2011 AACR.*

Introduction

Head and neck cancers typically include tumors of the oral cavity, pharynx, larynx, nose, nasal cavity and sinuses, and esophagus. This study focuses specifically on squamous cell cancers of the oral cavity, pharynx, and larynx.

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There were an estimated 49,260 new cases and 11,480 deaths from oropharyngeal and laryngeal cancer in the United States in 2010 (1). Globally in 2008, oral cavity tumors were among the top 10 incident cancers in men world wide, and the top 10 fatal cancers in men in developing countries (2).

Squamous cell carcinoma of the head and neck (SCCHN) incidence is higher in men than women, and in the United States, in African-Americans and the poor. Much of this disparity is due to higher incidence of laryngeal tumors among African-American men (3).

SCCHN is strongly associated with smoking tobacco products and drinking alcoholic beverages, and recently with human papillomavirus infection. It is estimated that 75% of SCCHN in the United States is due to cigarette smoking and alcohol consumption (4). The effect of these exposures varies by anatomic subsite, with smoking more associated with laryngeal tumors, and drinking with oral cavity tumors. However, only a small fraction of people exposed to these carcinogens will develop SCCHN,

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suggesting that other factors, including genetic, must be considered. Inherited genetic variation in alcohol metabolism has been suggested as a potentially important contributor to SCCHN risk. Investigation of the association between single-nucleotide polymorphisms (SNP) and SCCHN may help to identify high-risk groups and clarify carcinogenesis pathways.

Many studies of genes in the alcohol metabolism pathway (*ADH* family, *ALDH2*, *CYP2E1*) have been limited by sample size, and with one exception (5), none have included a significant percentage of African-Americans. Furthermore, few studies have examined the influence of genetic variation in oxidative stress pathways (*SOD*, *GPx*, *CAT*). We examined the association between SNPs and haplotypes of genes in the alcohol metabolism and oxidative stress pathways and SNP–alcohol interactions using data from a large NC population–based case–control study of SCCHN including 22% African-Americans.

Methods

Subject enrollment

The Carolina Head and Neck Cancer Epidemiology Study (CHANCE) is a population-based case–control study upon which these analyses are based (6).

All cases of squamous cell carcinoma of the oral cavity, pharynx, and larynx diagnosed in 46 NC counties between January 1, 2002, and February 28, 2006, were eligible for enrollment. Rapid case identification was conducted by the NC Central Cancer Registry. CHANCE cases included ICD-O-3 topography codes C0.00-C14.8 and C32.0-C32.9, excluding salivary gland (C07.9, C08.0-C08.9), nasopharynx (C11.0-C11.9), nasal cavity (C30.0), and nasal sinuses (C31.0-C31.9). ICD-O-3 morphology codes included were 8010/3, 8051/3, 8083/3, 8071/3, 8072/3, 8073/3, 8074/3, and 8076/3. Benign tumors, carcinomas *in situ*, papillary carcinomas, and adenoid carcinomas were excluded. We further excluded 21 lip cancers (C00.3-C00.9, C14.2), 46 of "other" race, and 96 without genotyping data, producing a study composition of 1,227 cases and 1,325 controls.

Potentially eligible controls from the same counties as cases were identified through NC Department of Motor Vehicles records. Controls were frequency matched to cases using random sampling with stratification on age, race, and sex.

Trained nurse-interviewers conducted an in-person interview with each subject. For this analysis only self-reported, nonproxy data were included. Questions were asked about demographics, tobacco use, drinking of alcoholic beverages, diet, oral health, medical history, and family history of cancer.

Blood samples were obtained by nurse-interviewers trained in phlebotomy. If the subject was not willing or able to consent to the blood draw, they were asked to contribute a buccal cell sample via mouth rinse.

Written informed consent was obtained from all subjects. The study was approved by the Biomedical Institutional Review Board at the University of North Carolina at Chapel Hill.

Outcome, exposure, and covariate measurement

Outcome. Case tumors were classified into anatomic subsites according to the following 5 ICD-O categories used by the International Head and Neck Cancer Epidemiology Consortium (ref. 7): (i) oral cavity: C02.0-C02.3, C03.0, C03.1, C03.9, C04.0, C04.1, C04.8, C04.9, C05.0, C06.0-C06.2, C06.8, and C06.9; (ii) oropharynx: C01.9, C02.4, C05.1, C05.2, C09.0, C09.1, C09.8, C09.9, C10.0-C10.4, C10.8, and C10.9; (iii) oral cavity–oropharynx–hypopharynx NOS: C02.8, C02.9, C05.8, C05.9, C14.0, C14.2, and C14.8; (iv) hypopharynx: C12.9, C13.0-C13.2, C13.8, and C13.9; and (v) larynx: C32.0-C32.3, and C32.8-C32.9.

Alcohol and tobacco use. Questions about alcohol use were designed to estimate lifetime history of consumption, and usual consumption of each beverage type, prior to the year before diagnosis. Questions asked about beer, wine, and hard liquor separately as follows: (i) Did you drink (beer/wine/hard liquor)? (ii) At what age did you start? (iii) At what age did you stop? (iv) For how many years did you drink (beer/wine/hard liquor) during this period? (v) How much (beer/wine/hard liquor) did you usually drink? Per day/week/month/year? and (vi) What size did you usually drink?

As frequency of drinking has shown stronger associations with SCCHN than duration (8), a single frequency measure that included all types of alcoholic beverages would have been optimal for estimating alcohol interaction with SNPs. Because this was unavailable in CHANCE, we instead derived a lifetime measure of alcohol intake, in milliliters, for beer, wine, and liquor combined. Using splines, we confirmed that tertiles best represented the risk associated with alcohol intake.

The primary tobacco exposure covariate selected was continuous duration of cigarette smoking. Dichotomous variables representing additional potential tobacco confounders were: ever use of non-cigarette tobacco, and ever-exposed to environmental tobacco smoke at work or at home.

SNPs and haplotypes. Seventy-five SNPs (69 tag SNPs, and 6 candidate SNPs found in prior studies to be associated with cancer incidence or survival, or alcohol dependence) were selected in 12 genes that are part of 2 metabolic pathways: *ADH1B*, *ADH1C*, *ADH4*, *ADH7*, *ALDH2*, and *CYP2E1* in the alcohol metabolism pathway in the upper aerodigestive tract; and *CAT*, *SOD1*, *SOD2*, *GPX1*, *GPX2*, and *GPX4* in the oxidative stress pathway. Tag SNPs, chosen to represent the genetic variation within each of the 12 candidate genes (gene and 2,000 bp upstream and downstream) were selected using the Genome Variation Server (9), using SNPs that were polymorphic in either CEU or YRI HapMap Release 2 (unrelated only), with the following parameters: allele frequency cutoff value of 10%, 0.8 R^2 threshold minimum for variations to belong to the same cluster, 85% minimum

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data coverage for tag SNPs, 70% minimal data coverage for a variation to be potentially clustered with others.

To control for potential population stratification, we selected 157 ancestry informative markers (AIM) to maximize (i) the difference in allele frequencies (delta) between European and African populations in the HapMap data (CEU vs. YRI), and (ii) the Fisher's information criterion (FIC). AIMs were prioritized on the basis of having the highest delta and FIC values in the following order: 90% European/10% African, 10% European/90% African, and 50% European/50% African. This allowed AIMs to represent the entire expected ancestral distribution of the study population. Individual estimates of percentage African ancestry were calculated from 145 successfully genotyped AIMs using maximum likelihood estimation methods previously described (10–12). AIMs were chosen to differentiate only between African and European ancestry, so individual ancestry estimates for the 2 groups sum to 1.0.

DNA was extracted from blood or buccal samples collected at time of interview. Genotyping was done by the University of North Carolina at Chapel Hill, Mammalian Genotyping Core Facility, using the Illumina GoldenGate Genotyping Assay with Sentrix Array matrix and 96-well standard microtiter plates.

Haplotypes using SNP data were constructed separately for African- and European-Americans using default D′ blocks in Haploview 4.2. The algorithm (13) constructs 95% confidence limits on D′ and each comparison is defined as "strong LD," "inconclusive," or "strong recombination." A block is created if 95% of informative comparisons are in "strong LD." Markers with minor allele frequency less than 5% are ignored. Assignment of most likely haplotype for individuals with ambiguous haplotype was done using an EM algorithm in haplo.stats (14), with minimum counts set to 10.

Socioeconomic status, oral health. Dichotomous variables representing additional potential confounders were: had health insurance on reference date, had a routine dental visit in the last 10 years, ever had a loose permanent tooth due to disease, ever used mouthwash, family history of SCCHN, household poverty as defined by federal guidelines, and education level.

Statistical analysis

ORs for the independent effects of SNPs and alcohol, and their interactive effects, were computed using conditional logistic regression implemented in SAS 9.2. ORs for the main effects of haplotypes were computed using unconditional logistic regression implemented in haplo.stats 1.4.4.

A dominant genetic model (at least one minor allele vs. referent of no minor alleles) was used for SNPs because for many SNPs, the number of subjects homozygous for the minor allele was too small to permit precise effect measurement.

Potential covariates were eliminated using stepwise backward elimination, comparing each reduced model with a full model that included all covariates listed in Table 1. No collinearity was noted between variables in the full model, with one exception as described below. If a covariate did not change the ln(OR) for any SNP by a difference of at least 0.10, it was eliminated from subsequent models. Final models for genetic main effects contained a single SNP or haplotype and duration of smoking as a continuous variable. Models estimating SNP–drinking interaction also included categorized lifetime ethanol consumption. We had insufficient power to detect haplotype–drinking interaction because haplotypes were constructed and analyzed separately for African- and European-Americans. The conditional logistic regression used for SNPs by definition takes into account the matching variables of age, sex, and race. The unconditional logistic regression models used for haplotypes (for each race separately) included, as covariates, sex, age, and their 2-way interaction. Ancestry was not important for the polymorphisms studied, probably because self-reported race was already included (as a matching variable). The ancestry variable also showed evidence of collinearity with race, so for these reasons and for parsimony's sake, ancestry was excluded from final models.

A Bonferroni correction was used to adjust P values and interaction contrast ratios (ICR) confidence intervals (CI) to control for type 1 error introduced by multiple statistical testing, for either 64 tests (for 64 SNPs) or for 12 or 13 tests (for haplotypes).

Departures from additive interaction were evaluated by computing ICRs and Bonferroni-corrected CIs. ICRs were calculated using cancer ORs of subjects in 3 categories: (i) the highest drinking category and no minor allele (OR_{01}); (ii) never-drinkers with at least one minor allele (OR_{10}); and (iii) subjects in the highest drinking category and at least one minor allele (OR_{11}), compared with never-drinkers homozygous for the major allele (i.e., the referent: $OR_{00} = 1.0$). ICR is calculated as follows: $ICR = OR_{11} - OR_{01} - OR_{10} + 1$. ICRs significantly different from zero indicate departure from additive interaction.

Results

Description of study population

Although controls were somewhat older and more likely to be female and European-American than cases (Table 1), the percentages of cases versus controls in each of the 28 age–sex–race cross-categories, as a proportion of the entire study population, differed by less than 2%. Compared with controls, cases smoked and drank more, and were poorer, less likely to have completed high school or have health insurance, less likely to have had a routine dental visit in the past 10 years, and more likely to have lost a permanent tooth to disease. Cases were also more likely to have been exposed to environmental tobacco smoke at home and work. Mean proportion of African ancestry was slightly higher in cases than controls.

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Table 1. Distribution of nongenetic variables in cases and controls

Variable	Cases ($n = 1,227$) n^a (%)	Controls ($n = 1,325$) n^a (%)	χ^2 or t test, unadjusted P-value
Age, y			
20–49	239 (19.5)	151 (11.4)	<0.0001
50–54	189 (15.4)	156 (11.8)	
55–59	207 (16.9)	199 (15.0)	
60–64	205 (16.7)	202 (15.2)	
65–69	168 (13.7)	237 (17.9)	
70–74	135 (11.0)	216 (16.3)	
75–80	84 (6.8)	164 (12.4)	
Sex			
Male	938 (76.4)	924 (69.7)	0.0001
Female	289 (23.6)	401 (30.3)	
Race			
European-American	922 (75.1)	1,074 (81.1)	0.0003
African-American	305 (24.9)	251 (18.9)	
Drinking (lifetime ethanol intake in mL)			<0.0001
Never-drinkers	117 (9.5)	280 (21.1)	
>0 to <134,699	210 (17.1)	467 (35.2)	
134,699 to <757,550	318 (25.9)	360 (27.2)	
757,550+	505 (41.2)	173 (13.1)	
Missing	77 (6.3)	45 (3.4)	
Smoking (duration in y)			<0.0001
0	160 (13.0)	497 (37.5)	
1–19	104 (8.5)	266 (20.1)	
20–39	435 (35.5)	314 (23.7)	
40–49	295 (24.0)	131 (9.9)	
50+	155 (12.6)	71 (5.4)	
Missing	78 (6.4)	46 (3.5)	
Poverty group (at or above, or below, federal poverty guideline)			<0.0001
≥Poverty guideline	816 (66.5)	1,088 (82.1)	
<Poverty guideline	356 (29.0)	187 (14.1)	
Had a routine dental visit in past 10 years?			<0.0001
Yes	781 (63.7)	1,115 (84.2)	
No	438 (35.7)	210 (15.8)	
Drank alcoholic beverages in prior 20 years?			0.8851
No	24 (2.0)	27 (2.0)	
Yes	1,202 (98.0)	1,298 (98.0)	
Ever exposed to environmental tobacco smoke at work			0.0024
No	316 (25.8)	414 (31.2)	
Yes	909 (74.1)	911 (68.8)	
Ever exposed to environmental tobacco smoke at home			<0.0001
No	399 (32.5)	592 (44.7)	
Yes	827 (67.4)	732 (55.2)	
Ever used non-cigarette tobacco products			0.1165
No	754 (61.5)	854 (64.5)	
Yes	473 (38.5)	471 (35.5)	
Had health insurance at reference date			<0.0001
Yes	1,068 (87.0)	1,250 (94.3)	
No	154 (12.6)	74 (5.6)	
Highest education level attained			<0.0001
≥High school	828 (67.5)	1,123 (84.8)	
<High school	399 (32.5)	202 (15.2)	

(Continued on the following page)

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Table 1. Distribution of nongenetic variables in cases and controls (Cont'd)

Variable	Cases ($n = 1,227$) n^a (%)	Controls ($n = 1,325$) n^a (%)	χ^2 or t test, unadjusted P-value
Ever had loose permanent tooth due to disease			<0.0001
No	765 (62.3)	1,018 (76.8)	
Yes	455 (37.1)	305 (23.0)	
Ever regularly used mouthwash			0.8572
No	502 (40.9)	549 (41.4)	
Yes	719 (58.6)	775 (58.5)	
Family history of SCCHN among first-degree relatives			0.3848
No	1,206 (98.3)	1,296 (97.8)	
Yes	21 (1.7)	29 (2.2)	
Mean African ancestry, %	23.8	19.7	0.0008

[a]Frequencies for all variables may not sum to the total number of cases and controls, due to missing values.

Sixty-four of 75 SNPs (45 alcohol metabolism, 19 oxidative stress) were successfully genotyped. Assay intensity data and genotype cluster images for all SNPs were individually reviewed; as a result, 9 of the original 75 tag SNPs and 12 AIMs (9% of SNPs) were excluded due to inadequate signal or indistinguishable genotype clusters. Blind duplicates of 109 samples were genotyped to verify call reliability; none of our SNPs were discrepant. Two of the original 75 tag SNPs were judged to be out of Hardy–Weinberg Equilibrium (SAS PROC ALLELE) in both European- and African-American controls due to an exact P value <0.001, and were eliminated from analysis.

There were no large differences in allele frequencies between cases and controls, when stratified by race (Supplementary Table S1). However there are large allele frequency differences between African- and European-Americans.

Cancer risk from alcohol consumption

The odds of developing SCCHN increase monotonically as lifetime alcohol consumption increases (Table 2). Subjects in the lowest consumption category experienced reduced SCCHN odds compared with nondrinkers (OR = 0.8; 95% CI, 0.6–1.0), driven largely by laryngeal and oral cavity tumors (OR = 0.7; 95% CI, 0.4–1.1 and OR = 0.4; 95% CI, 0.2–0.9, respectively).

Successively higher levels of alcohol consumption were associated with increasing odds. The middle tertile of lifetime consumption was associated with 30% higher SCCHN odds than never-drinkers, and the highest tertile of consumption with tripled odds. In the highest drinking category, all subsites experienced significantly increased odds: doubled odds of laryngeal cancer, and tripled or greater odds for oropharyngeal and oral cavity tumors.

Cancer risk from genetic variants

None of the SNP associations with SCCHN or any of the subsite cancers had a significant Bonferroni-corrected P value, although 5 SNPs in *ADH1B*, *ALDH2*, and *SOD2* showed evidence of reduced or increased cancer ORs overall and in oral cavity, laryngeal, and hypopharyngeal subsites (Table 3; remaining subsite effects in Supplementary Table S2). In *ADH1B*, the rs1229984 A allele was associated with 30% decreased SCCHN odds, and the rs17028834 C allele with 50% increased odds of laryngeal tumors. In *ALDH2*, the rs2238151 C allele was associated with 10% increased odds of SCCHN, driven largely by 20% increased risk of laryngeal tumors. In *SOD2*, the rs4342445 A allele was associated with 30% greater odds for oral cavity tumors, and the rs5746134 T allele with doubled odds for hypopharyngeal cancer.

Linkage disequilibrium was strong among SNPs within genes, not between genes, so haplotypes included only SNPs within the same gene (Supplementary Table S3). Four haplotypes in *ALDH2*, *CYP2E1*, *GPX2*, and *SOD1* were associated with SCCHN, either in European-Americans or African-Americans, or both (Table 4). One *GPX2* haplotype was significantly associated with 30% decreased odds of SCCHN in European-Americans. An *ALDH2* haplotype was associated with 50% reduced odds in African-Americans and a *CYP2E1* haplotype was associated with 30% reduced odds in European-Americans. The *SOD1* AGGC haplotype was associated with increased odds in European-Americans and reduced odds in African-Americans.

To examine the potential impact of multiple at-risk alcohol metabolism alleles, we counted the number of previously studied risk alleles (0–4) for each individual, including these alleles: *ADH1B* rs1229984 'G', *ADH1C* rs1693482 'T', *ADH7* rs1573496 'G', and *CYP2E1* rs3813867 'C'. The numbers of risk alleles was not associated with an increased or decreased risk of SCCHN (data not shown).

Cancer risk from alcohol interaction with SNPs

Four SNPs showed evidence of synergistic additive interaction with alcohol consumption (Table 5). All

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Table 2. Effect of lifetime alcohol consumption on odds of developing cancer

	SCCHN (all 5 sub sites combined)		Oral cavity cancer		Oropharyngeal cancer		Oral cavity, oropharyngeal, hypopharyngeal cancer NOS		Hypopharyngeal cancer		Laryngeal cancer	
Lifetime alcohol Consumption, mL	No. of cases/controls[a]	Adjusted OR[b] (95% CI)	No. of cases/controls	Adjusted OR[b] (95% CI)	No. of cases/controls	Adjusted OR[b] (95% CI)	No. of cases/controls	Adjusted OR[b] (95% CI)	No. of cases/controls	Adjusted OR[b] (95% CI)	No. of cases/controls	Adjusted OR[b] (95% CI)
Missing	74/43		6/43		22/43		15/43		3/43		28/43	
0	117/280	1.00 (ref)	22/280	1.00 (ref)	27/280	1.00 (ref)	23/280	1.00 (ref)	1/280	1.00 (ref)	44/280	1.00 (ref)
>0–133,294	209/466	0.75 (0.56–1.02)	19/466	0.45 (0.23–0.89)	69/466	0.87 (0.53–1.44)	48/466	0.93 (0.54–1.62)	5/466	2.25 (0.26–19.84)	68/466	0.67 (0.42–1.08)
133,294–757,550	318/360	1.29 (0.95–1.76)	41/360	1.28 (0.68–2.41)	94/360	1.47 (0.89–2.45)	51/360	1.48 (0.83–2.64)	9/360	5.13 (0.61–43.04)	123/360	1.25 (0.78–2.00)
757,550+	505/173	3.22 (2.29–4.52)	84/173	5.34 (2.67–10.67)	120/173	3.47 (2.00–6.04)	86/173	4.49 (2.40–8.39)	36/173	28.74 (3.42–241.40)	179/173	2.26 (1.38–3.70)

[a]Cases and controls do not sum to 1,227 and 1,325, respectively, because 4 cases and 3 controls are missing information on duration of cigarette smoking.

[b]Conditional logistic regression models for estimating main effects of categorized lifetime ethanol consumption were conditioned on sex, race, and age category, and adjusted for continuous smoking duration rounded to whole years.

met the following 2 characteristics: (i) statistically significant or near-significant Bonferroni-corrected CI for ICR (64 tests), and (ii) at least 10 cases and 10 controls in each of the 3 comparison groups OR_{01}, OR_{10}, and OR_{11}. For example, heavy drinkers carrying the C allele of rs2238151 in *ALDH2* showed statistically significant evidence of synergistic additive interaction. Also the T allele at rs1159918 in *ADH1B*, the A allele at rs1154460 in *ADH7*, and the T allele at rs2249695 in *CYP2E1* showed some evidence for synergistic additive interaction between alcohol consumption and SNP. (Evaluations of additive interaction with alcohol for remaining SNPs can be found in Supplementary Table S4.)

No interactions with alcohol were detected for anatomic subsites.

SNP effects by race

SNP effect estimates were similar in European- and African-Americans, with a few exceptions. Two SNPs in *SOD1* (rs10432782, rs2070424) were associated with decreased odds of SCCHN in African-Americans and increased odds in European-Americans (Supplementary Table S5; rs10432782, OR = 0.65; 95% CI, 0.42–1.00 in African-Americans, OR = 1.35; 95% CI, 1.07–1.71 in European-Americans; rs2070424, OR = 0.52; 95% CI, 0.33–0.83 in African-Americans, OR = 1.47; 95% CI, 1.10–1.97 in European-Americans). Three additional SNPs, which had sufficient frequency of the minor allele in both races, showed evidence of risk differences by race (*ADH1B* rs1693457, *ADH4* rs10017466, *SOD1* rs4998557) though CIs for races overlapped (Supplementary Table S5). The magnitude of the joint effect for the 4 SNPs found to interact additively with alcohol exposure did not differ between races (data not shown).

Discussion

Alcohol consumption

Most studies report a strong dose–response relationship between higher levels of drinking, both in lifetime frequency of drinking (e.g., drinks per day) and lifetime alcohol intake (e.g., milliliters of ethanol), and increased SCCHN risk. However, the type of alcohol beverage most strongly associated with cancer risk varies substantially by study, and some studies suggest that the most common alcoholic beverage in the geographic region studied produces the highest cancer risk (15). There is some evidence that moderate levels of wine consumption produce lower risk than beer and liquor (comparing 16–30 ethanol-standardized drinks per week of each type), but above 30 drinks per week, all types are associated with increased risk (15).

We found a general pattern of association with alcohol intake that is consistent with previous studies (7), with monotonically increasing cancer risk as lifetime

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consumption increases. Beer and liquor accounted for about 90% of lifetime alcohol consumption in our study population, and those beverages were associated with higher cancer risk than wine consumption (data not shown). This is consistent with the hypothesis that the most commonly drunk alcoholic beverages are associated with the highest risk.

Alcohol metabolism genes

ADH, ALDH. Variant *ADH* and *ALDH* alleles coding for either superactive or inactive subunits of ADH and ALDH isozymes are common. Numerous studies in Asian populations have reported an association between several presumably functional variants in *ADH1B*, *ADH1C*, *ADH4*, *ADH7*, and *ALDH2* and SCCHN incidence (16–23). However, these studies lacked sufficient power to consistently detect interaction between gene and alcohol drinking. In recent years, these variants and others were investigated in larger studies of Europeans, Latin-Americans, and Indians with similar findings (24–33). However, only a few smaller studies examined risk in European-Americans and African-Americans (5, 34–37), and those included very small numbers of African-Americans.

We discovered an association between rs1229984 in *ADH1B* and SCCHN odds ($OR_{AA + AG \text{ vs. } GG} = 0.72$; 95% CI, 0.57–0.91). It is the same direction of effect for the A allele as reported in a Japanese study (ref. 21; $OR_{GG + GA \text{ vs. } AA} = 2.20$; 95% CI, 1.46–3.32) and in European-Caucasians and Latin-Americans (ref. 29; $OR_{AA + GA \text{ vs. } GG} = 0.56$; 95% CI, 0.47–0.66), but is the reverse of the effect reported in a few other studies [(18, 27, 28) e.g., (28)]: $OR_{GG + GA \text{ vs. } AA} = 0.36$; 95% CI, 0.17–0.77). A recent INHANCE GWAS (38) reported a replicated association of 5 SNPs with SCCHN and esophageal cancer, including rs1229984, for which the A allele under a log-additive genetic model was associated with reduced odds in both the discovery (OR = 0.52; 95% CI, 0.43–0.64) and the replication phases (OR = 0.68; 95% CI, 0.60–0.78). The GWAS replication sample included 2,027 CHANCE subjects as 10% of the replication sample. In our study, only 6 African-American subjects carried the A allele, compared with 104 European-Americans, so most of the effect we observed for rs1229984 occurred in European-Americans.

We found no effect on SCCHN risk of the rs1693482 "slow" allele in *ADH1C* ($OR_{TT + TC \text{ vs. } CC} = 1.05$; 95% CI, 0.95–1.15). The 2 largest studies of this SNP and SCCHN in European-Caucasians (28) and European-Caucasians and Latin-Americans (29) found 20% to 50% increased odds associated with this allele. Also, all 4 studies of rs698 "slow" or G allele in Brazilian, Japanese, European-American, and Latin-American populations (21, 27–29) reported evidence of 16% to 38% increased odds. In CHANCE, rs1631460 is in high LD ($r^2 = 0.95$) with rs698 in both CEU and YRI HapMap populations, but we found no association between it and SCCHN.

No *ADH4* and *ADH7* SNPs were associated with SCCHN, including the rs1573496 C allele in *ADH7*. This is in contrast to the one study that investigated this allele and found it to be associated with 30% reduced odds in Europeans and Latin-Americans (29).

No *ALDH2* SNPs were associated with SCCHN, and a possible haplotype association was present only in African-Americans (OR = 0.5; 95% CI, 0.3–0.8). Previous studies of rs886205, an *ALDH2* SNP that is polymorphic in Europeans, found conflicting results of no association and increased association for the G allele (26, 28).

Our findings may differ from those previously reported due to differences in sample size, the specific population studied, and the composition of tumor subsites included.

Gene interaction with alcohol

We discovered evidence of synergistic additive interaction with alcohol of several SNPs in alcohol metabolism pathway genes, although the SNPs we identified were different from those previously reported in the literature. Specifically, we found 2 SNPs in *ADH1B* and *ADH7*—rs1159918 and rs1154460, respectively—that seem to interact with alcohol. We also found one previously unstudied *ALDH2* SNP, rs2238151, that showed evidence of additive interaction ($OR_{11actual} = 3.3$ vs. $OR_{11expected} = 1.4$). Whereas previous studies reported that rs1229984 in *ADH1B*, rs4148887 in *ADH4*, rs1573496 in *ADH7*, and rs886205, rs441 (both in high LD with our SNP rs4767939), and rs440 in *ALDH2* interacted with alcohol drinking (16, 18, 27–29), we did not find evidence for an interaction with these SNPs, probably because we measured alcohol consumption using lifetime alcohol intake instead of drinking frequency.

We also found evidence for synergistic additive interaction for *CYP2E1* rs2249695 with alcohol. A recent linkage and association study (39) identified that SNP, among others, to be associated with "tipsiness," or quick response to alcohol challenge. In CHANCE, the T allele (Table 5, last row) was protective in never-drinkers ($OR_{10} = 0.6$; 95% CI, 0.4–0.9) but was associated with 70% greater than expected risk in the heaviest drinkers.

Oxidative stress genes

We found 2 previously unstudied SNPs in *SOD2* to be associated with subsite tumors: rs4342445 with oral cavity, and rs5746134 with hypopharynx. One *SOD1* haplotype was associated with SCCHN risk in both races, albeit in different directions, due to the effect of multiple individual SNP effects that differed by race in that gene. Finally, we found a *GPX2* haplotype to be associated with reduced SCCHN risk in European-Americans only. This may indicate that the haplotype is in high linkage disequilibrium with an unmeasured causal polymorphism in European-Americans but not in African-Americans.

Table 3. SNP effects on odds of developing cancer (dominant genetic model)

			SCCHN (all 5 anatomic subsites, combined)				Oral cavity cancer				Hypopharyngeal cancer				Laryngeal cancer			
			No. of cases/controls[a]		Adjusted OR[b] (95% CI)	P^c	No. of cases/controls		Adjusted OR[b] (95% CI)	P^c	No. of cases/controls		Adjusted OR[b] (95% CI)	P^c	No. of cases/controls		Adjusted OR[b] (95% CI)	P^c
Gene	SNP	Major/ minor alleles	Homozygous for major allele	One or two copies of minor allele			Homozygous for major allele	One or two copies of minor allele			Homozygous for major allele	One or two copies of minor allele			Homozygous for major allele	One or two copies of minor allele		
Alcohol metabolism genes																		
ADH1B	rs12507573	C/A	371/386	852/935	0.96 (0.87–1.06)	1.00	57/386	115/935	0.94 (0.78–1.14)	1.00	15/386	39/935	1.03 (0.74–1.42)	1.00	137/386	305/935	0.94 (0.82–1.08)	1.00
	rs1042026	A/G	712/744	511/578	1.00 (0.91–1.10)	1.00	94/744	78/578	1.06 (0.88–1.26)	1.00	36/744	18/578	0.89 (0.65–1.22)	1.00	264/744	178/578	0.96 (0.84–1.10)	1.00
	rs7673353	C/T	1,124/1,239	99/83	1.01 (0.83–1.23)	1.00	161/1,239	11/83	0.88 (0.60–1.30)	1.00	48/1,239	6/83	0.84 (0.51–1.40)	1.00	402/1,239	40/83	1.04 (0.79–1.36)	1.00
	rs17028834	T/C	1,138/1,265	85/57	1.28 (1.03–1.59)	1.00	159/1,265	13/57	1.50 (1.01–2.25)	1.00	48/1,265	6/57	1.71 (1.00–2.95)	1.00	408/1,265	34/57	1.49 (1.11–2.01)	**0.54**
	rs1693457	T/C	803/860	419/461	1.01 (0.92–1.11)	1.00	114/860	58/461	0.99 (0.82–1.19)	1.00	36/860	18/461	0.94 (0.69–1.28)	1.00	294/860	148/461	1.02 (0.89–1.17)	1.00
	rs1229984[d]	G/A	1,192/1,243	31/79	0.72 (0.57–0.91)	**0.35**	169/1,243	3/79	0.63 (0.35–1.15)	1.00	53/1,243	1/79	0.62 (0.22–1.70)	1.00	429/1,243	13/79	0.79 (0.57–1.12)	1.00
	rs1159918	G/T	402/468	821/854	1.07 (0.97–1.18)	1.00	60/468	112/854	1.03 (0.86–1.24)	1.00	14/468	40/854	1.17 (0.83–1.64)	1.00	154/468	288/854	1.05 (0.91–1.21)	1.00
	rs1229982	G/T	699/775	524/547	1.00 (0.91–1.10)	1.00	97/775	75/547	0.95 (0.80–1.14)	1.00	35/775	19/547	0.80 (0.59–1.09)	1.00	241/775	201/547	1.05 (0.92–1.19)	1.00
ADH1C	rs2298753	T/C	1,002/1,117	221/205	1.12 (1.00–1.27)	1.00	140/1,117	32/205	1.12 (0.89–1.41)	1.00	46/1,117	8/205	1.16 (0.78–1.75)	1.00	366/1,117	76/205	1.10 (0.93–1.31)	1.00
	rs1614972	C/T	539/585	684/737	1.00 (0.92–1.09)	1.00	85/585	87/737	0.93 (0.78–1.10)	1.00	26/585	28/737	0.83 (0.61–1.11)	1.00	192/585	250/737	0.96 (0.85–1.10)	1.00
	rs1391088	C/A	1,045/1,119	178/203	1.01 (0.89–1.14)	1.00	142/1,119	30/203	1.07 (0.85–1.34)	1.00	50/1,119	4/203	0.71 (0.41–1.20)	1.00	381/1,119	61/203	1.05 (0.88–1.26)	1.00
	rs1693482[e]	C/T	527/585	694/735	1.05 (0.95–1.15)	1.00	75/585	97/735	1.00 (0.83–1.20)	1.00	24/585	30/735	1.18 (0.87–1.60)	1.00	186/585	256/735	1.09 (0.95–1.25)	1.00
	rs1631460	C/G	519/574	703/748	1.04 (0.95–1.14)	1.00	74/574	97/748	0.99 (0.83–1.19)	1.00	24/574	30/748	1.17 (0.86–1.58)	1.00	184/574	258/748	1.08 (0.94–1.23)	1.00
	rs11936869	C/G	611/645	612/676	0.98 (0.90–1.07)	1.00	90/645	82/676	0.96 (0.81–1.14)	1.00	28/645	26/676	0.86 (0.64–1.16)	1.00	221/645	221/676	0.93 (0.82–1.06)	1.00
ADH4	rs29001227	A/T	1,119/1,253	104/69	1.25 (1.02–1.53)	1.00	157/1,253	15/69	1.31 (0.90–1.91)	1.00	48/1,253	6/69	1.38 (0.81–2.34)	1.00	401/1,253	41/69	1.39 (1.05–1.84)	1.00
	rs1126672	C/T	720/758	503/564	1.01 (0.92–1.10)	1.00	93/758	79/564	1.08 (0.90–1.28)	1.00	38/758	16/564	0.82 (0.60–1.13)	1.00	257/758	185/564	1.06 (0.93–1.21)	1.00
	rs4699710	T/C	640/684	583/638	1.01 (0.93–1.11)	1.00	77/684	95/638	1.12 (0.94–1.33)	1.00	34/684	20/638	0.86 (0.64–1.16)	1.00	230/684	212/638	1.05 (0.93–1.20)	1.00
	rs10017466	T/C	556/632	666/690	1.05 (0.97–1.15)	1.00	67/632	105/690	1.16 (0.97–1.38)	1.00	30/632	24/690	0.90 (0.68–1.20)	1.00	196/632	246/690	1.12 (0.99–1.27)	1.00
	rs1800759	C/A	357/412	866/910	1.02 (0.92–1.12)	1.00	46/412	126/910	1.09 (0.90–1.33)	1.00	13/412	41/910	1.05 (0.75–1.48)	1.00	122/412	320/910	1.09 0.94–1.25)	1.00
	rs1800761	G/A	750/830	473/492	1.02 (0.93–1.12)	1.00	112/830	60/492	0.92 (0.77–1.10)	1.00	35/830	19/492	0.90 (0.66–1.22)	1.00	276/830	166/492	0.99 (0.87–1.13)	1.00
	rs3762894	T/C	839/906	384/416	0.98 (0.89–1.08)	1.00	126/906	46/416	0.89 (0.74–1.08)	1.00	38/906	16/416	0.89 (0.65–1.23)	1.00	307/906	135/416	0.94 (0.82–1.08)	1.00
ADH7	rs284787	C/T	739/817	484/505	1.06 (0.96–1.16)	1.00	112/817	60/505	0.94 (0.79–1.13)	1.00	29/817	25/505	1.18 (0.88–1.59)	1.00	264/817	178/505	1.09 (0.96–1.25)	1.00
	rs894369	C/G	832/849	391/472	0.92 (0.84–1.01)	1.00	116/849	56/472	0.96 (0.80–1.15)	1.00	42/849	12/472	0.75 (0.54–1.05)	1.00	304/849	138/472	0.93 (0.81–1.06)	1.00
	rs17588403	T/A	837/887	386/435	0.96 (0.87–1.05)	1.00	116/887	56/435	0.95 (0.79–1.14)	1.00	37/887	17/435	0.94 (0.69–1.28)	1.00	297/887	145/435	0.96 (0.84–1.10)	1.00
	rs1154454	T/C	756/808	467/514	0.94 (0.86–1.04)	1.00	117/808	55/514	0.80 (0.66–0.98)	1.00	37/808	17/514	0.69 (0.49–0.97)	1.00	260/808	182/514	1.00 (0.87–1.15)	1.00
	rs1154456	T/C	577/629	646/693	1.03 (0.94–1.13)	1.00	86/629	86/693	0.97 (0.81–1.15)	1.00	26/629	28/693	1.04 (0.78–1.40)	1.00	207/629	235/693	1.07 0.94–1.21)	1.00
	rs1154460	G/A	329/377	891/944	1.04 (0.95–1.15)	1.00	49/377	123/944	1.03 (0.85–1.25)	1.00	13/377	41/944	1.11 (0.80–1.55)	1.00	118/377	322/944	1.12 (0.97–1.29)	1.00
	rs971074	G/A	943/1,027	280/295	1.01 (0.91–1.12)	1.00	131/1,027	41/295	1.09 (0.89–1.34)	1.00	39/1,027	15/295	1.14 (0.83–1.58)	1.00	348/1,027	94/295	1.00 (0.85–1.16)	1.00
	rs1573496[d]	C/G	1,056/1,117	166/205	0.99 (0.87–1.12)	1.00	147/1,117	25/205	1.09 (0.86–1.39)	1.00	48/1,117	6/205	0.91 (0.57–1.44)	1.00	383/1,117	59/205	1.02 (0.85–1.23)	1.00
ALDH2	rs4767939	A/G	694/806	528/516	1.08 (0.98–1.18)	1.00	101/806	71/516	1.04 (0.87–1.25)	1.00	29/806	25/516	1.10 (0.81–1.49)	1.00	238/806	204/516	1.12 (0.98–1.28)	1.00
	rs2238151	T/C	364/482	855/837	1.13 (1.03–1.25)	0.84	51/482	121/837	1.18 (0.97–1.44)	1.00	16/482	38/837	1.00 (0.70–1.42)	1.00	117/482	323/837	1.24 (1.07–1.43)	**0.32**
	rs7312055	G/A	1,081/1,201	142/121	0.93 (0.78–1.12)	1.00	150/1,201	22/121	0.95 (0.67–1.34)	1.00	44/1,201	10/121	0.92 (0.57–1.50)	1.00	390/1,201	52/121	0.87 (0.67–1.12)	1.00
	rs2158029	G/A	1,166/1,269	57/53	0.95 (0.75–1.19)	1.00	165/1,269	7/53	0.76 (0.48–1.21)	1.00	50/1,269	4/53	0.98 (0.52–1.82)	1.00	419/1,269	23/53	0.99 (0.72–1.36)	1.00
	rs16941667	C/T	985/1,097	238/224	1.10 (0.98–1.23)	1.00	142/1,097	30/224	1.11 (0.88–1.40)	1.00	41/1,097	13/224	1.24 (0.88–1.75)	1.00	347/1,097	95/224	1.17 (0.99–1.37)	1.00
	rs16941669	T/G	984/1,084	239/236	1.06 (0.95–1.19)	1.00	134/1,084	38/236	1.20 (0.97–1.48)	1.00	45/1,084	9/236	1.00 (0.68–1.47)	1.00	358/1,084	84/236	1.10 (0.93–1.29)	1.00
CYP2E1	rs3813865	G/C	1,098/1,215	125/106	1.08 (0.92–1.27)	1.00	155/1,215	17/106	1.16 (0.85–1.58)	1.00	48/1,215	6/106	1.09 (0.67–1.77)	1.00	393/1,215	49/106	1.11 (0.89–1.38)	1.00
	rs3813867[d]	G/C	1,139/1,237	83/84	0.97 (0.82–1.16)	1.00	160/1,237	12/84	0.98 (0.70–1.38)	1.00	51/1,237	3/84	0.87 (0.47–1.62)	1.00	405/1,237	36/84	1.09 (0.86–1.39)	1.00
	rs8192772	T/C	1,023/1,114	199/208	1.07 (0.95–1.21)	1.00	142/1,114	30/208	1.09 (0.87–1.38)	1.00	44/1,114	9/208	1.11 (0.75–1.64)	1.00	363/1,114	79/208	1.14 (0.96–1.35)	1.00
	rs915908	G/A	974/1,006	249/316	0.93 (0.84–1.04)	1.00	139/1,006	33/316	0.92 (0.74–1.14)	1.00	46/1,006	8/316	0.77 (0.51–1.15)	1.00	362/1,006	80/316	0.87 (0.74–1.02)	1.00
	rs915909	C/T	1,164/1,271	58/46	1.00 (0.79–1.27)	1.00	164/1,271	7/46	0.88 (0.54–1.44)	1.00	50/1,271	4/46	1.09 (0.60–1.99)	1.00	421/1,271	21/46	0.92 (0.66–1.28)	1.00
	rs7092584	C/T	959/1,052	262/269	1.05 (0.95–1.17)	1.00	131/1,052	41/269	1.12 (0.91–1.38)	1.00	45/1,052	8/269	0.86 (0.58–1.28)	1.00	340/1,052	101/269	1.11 (0.96–1.30)	1.00
	rs743535	C/T	966/1,071	248/243	1.06 (0.95–1.19)	1.00	134/1,071	38/243	1.09 (0.88–1.35)	1.00	40/1,071	13/243	1.18 (0.84–1.66)	1.00	342/1,071	96/243	1.10 (0.94–1.29)	1.00
	rs2249695	C/T	622/682	600/639	0.93 (0.85–1.03)	1.00	87/682	84/639	0.93 (0.77–1.12)	1.00	24/682	30/639	0.95 (0.68–1.33)	1.00	223/682	219/639	0.93 (0.81–1.08)	1.00
	rs28969387	A/T	1,198/1,302	25/20	0.98 (0.70–1.37)	1.00	170/1,302	2/20	0.69 (0.31–1.56)	1.00	54/1,302	0/20		1.00	429/1,302	13/20	1.08 (0.70–1.68)	1.00
	rs11101812	T/C	1,194/1,300	26/20	1.11 (0.79–1.56)	1.00	170/1,300	2/20	0.79 (0.35–1.77)	1.00	51/1,300	3/20	1.79 (0.86–3.71)	1.00	427/1,300	14/20	1.38 (0.89–2.13)	1.00

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Table 3. SNP effects on odds of developing cancer (dominant genetic model) (Cont'd)

Gene	SNP	Major/ minor alleles	SCCHN (all 5 anatomic subsites, combined)				Oral cavity cancer				Hypopharyngeal cancer				Laryngeal cancer			
			No. of cases/controls[a]		Adjusted OR[b] (95% CI)	P^c	No. of cases/controls		Adjusted OR[b] (95% CI)	P^c	No. of cases/controls		Adjusted OR[b] (95% CI)	P^c	No. of cases/controls		Adjusted OR[b] (95% CI)	P^c
			Homozygous for major allele	One or two copies of minor allele			Homozygous for major allele	One or two copies of minor allele			Homozygous for major allele	One or two copies of minor allele			Homozygous for major allele	One or two copies of minor allele		
Oxidative stress metabolism genes																		
CAT	rs1049982[d]	C/T	521/589	700/726	1.02 (0.93–1.11)	1.00	78/589	94/726	0.95 (0.80–1.14)	1.00	27/589	26/726	0.82 (0.61–1.11)	1.00	182/589	260/726	1.05 (0.92–1.19)	1.00
GPX1	rs8179172	T/A	1,172/1,271	51/51	0.92 (0.73–1.17)	1.00	166/1,271	6/51	0.86 (0.52–1.41)	1.00	51/1,271	3/51	0.67 (0.32–1.39)	1.00	423/1,271	19/51	0.86 (0.62–1.19)	1.00
	rs1800668	C/T	635/672	588/646	1.04 (0.95–1.14)	1.00	84/672	88/646	1.14 (0.96–1.35)	1.00	29/672	25/646	1.01 (0.76–1.35)	1.00	237/672	205/646	1.03 (0.91–1.17)	1.00
	rs3811699	A/G	609/649	614/673	1.04 (0.95–1.13)	1.00	77/649	95/673	1.16 (0.98–1.38)	1.00	26/649	28/673	1.07 (0.81–1.43)	1.00	230/649	212/673	1.02 (0.90–1.16)	1.00
	rs3448	C/T	648/729	575/593	1.03 (0.95–1.13)	1.00	96/729	76/593	1.02 (0.86–1.21)	1.00	27/729	27/593	1.18 (0.88–1.58)	1.00	222/729	220/593	1.12 (0.99–1.28)	1.00
GPX2	rs11623705	G/T	996/1,069	227/253	1.00 (0.89–1.12)	1.00	139/1,069	33/253	0.99 (0.79–1.23)	1.00	44/1,069	10/253	1.01 (0.70–1.47)	1.00	360/1,069	82/253	1.01 (0.86–1.19)	1.00
	rs2412065	G/C	694/733	529/589	0.94 (0.86–1.03)	1.00	96/733	76/589	0.94 (0.79–1.13)	1.00	32/733	22/589	0.81 (0.60–1.10)	1.00	251/733	191/589	0.97 (0.85–1.11)	1.00
	rs2737844	C/T	493/511	725/806	0.89 (0.81–0.99)	1.00	72/511	98/806	0.86 (0.71–1.04)	1.00	20/511	34/806	0.87 (0.63–1.21)	1.00	177/511	265/806	0.91 (0.80–1.05)	1.00
GPX4	rs757229[d]	G/C	300/358	922/963	1.03 (0.93–1.14)	1.00	39/358	133/963	1.08 (0.88–1.32)	1.00	13/358	41/963	0.93 (0.66–1.31)	1.00	103/358	339/963	1.04 (0.90–1.21)	1.00
SOD1	rs11910115	A/C	1,161/1,257	62/65	0.93 (0.74–1.17)	1.00	165/1,257	7/65	0.76 (0.48–1.20)	1.00	51/1,257	3/65	0.80 (0.41–1.58)	1.00	418/1,257	24/65	0.94 (0.69–1.30)	1.00
	rs4998557	G/A	829/959	393/360	1.09 (0.99–1.21)	1.00	123/959	49/360	0.98 (0.80–1.21)	1.00	31/959	23/360	1.28 (0.93–1.78)	1.00	288/959	154/360	1.17 (1.01–1.36)	1.00
	rs10432782	T/G	874/992	348/329	1.07 (0.97–1.18)	1.00	131/992	41/329	0.93 (0.76–1.15)	1.00	34/992	20/329	1.24 (0.90–1.71)	1.00	306/992	136/329	1.13 (0.97–1.31)	1.00
	rs2070424	A/G	1,003/1,120	220/202	1.06 (0.94–1.20)	1.00	142/1,120	30/202	1.02 (0.80–1.30)	1.00	46/1,120	8/202	0.85 (0.55–1.31)	1.00	354/1,120	88/202	1.09 (0.92–1.29)	1.00
	rs1041740	C/T	682/705	541/617	0.98 (0.90–1.07)	1.00	95/705	77/617	1.00 (0.84–1.20)	1.00	36/705	18/617	0.82 (0.60–1.12)	1.00	250/705	192/617	0.97 (0.85–1.11)	1.00
SOD2	rs4342445	G/A	752/850	471/472	1.11 (1.02–1.22)	1.00	95/850	77/472	1.33 (1.12–1.59)	**0.09**	42/850	12/472	0.84 (0.60–1.18)	1.00	268/850	174/472	1.16 (1.01–1.32)	1.00
	rs2842980	A/T	715/807	508/515	1.02 (0.94–1.12)	1.00	97/807	75/515	1.05 (0.88–1.25)	1.00	31/807	23/515	0.98 (0.73–1.31)	1.00	269/807	173/515	0.97 (0.85–1.10)	1.00
	rs8031	T/A	387/382	836/940	0.98 (0.89–1.08)	1.00	52/382	120/940	1.00 (0.83–1.21)	1.00	16/382	38/940	1.12 (0.80–1.55)	1.00	140/382	302/940	1.02 (0.89–1.17)	1.00
	rs5746134	C/T	1,125/1,257	98/65	1.20 (0.98–1.48)	1.00	162/1,257	10/65	0.98 (0.64–1.50)	1.00	43/1,257	11/65	2.14 (1.25–3.67)	**0.34**	409/1,257	33/65	1.13 (0.84–1.50)	1.00
	rs2758331	C/A	422/419	801/903	0.99 (0.90–1.09)	1.00	61/419	111/903	0.99 (0.82–1.20)	1.00	17/419	37/903	1.22 (0.88–1.70)	1.00	150/419	292/903	1.04 (0.91–1.20)	1.00

[a]Cases and controls do not sum to 1,227 and 1,325, respectively, because 4 cases and 3 controls are missing information on duration of cigarette smoking, and because a few subjects lack genotype information for some SNPs.

[b]Conditional logistic regression models for estimating main effect of each SNP were conditioned on sex, race, and age category, and adjusted for continuous smoking duration rounded to whole years. ORs are for those with one or more copies of the minor allele versus the referent group of those homozygous for the major allele (dominant genetic model).

[c]Bonferroni corrected for 64 statistical tests. *P*-values highlighted in BOLD are near statistically significant after Bonferroni correction.

[d]Not a tag SNP; included in analyses because previous studies had examined it. rs1229984 and rs1573496 have missense variants; rs3813867 and rs757229 are located 5′ near the gene; rs1049982 has a synonymous variant and is located in exon 1.

[e]Selected as a tag SNP, but had also been studied in the literature because it has a missense variant.

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Table 4. Selected haplotype[a] main effects on SCCHN risk, additive genetic model

Gene (haplotype definition)	Haplotype	Race[b] (% prevalence)	OR (95% CI)[c]
ALDH2 (rs4767939, rs2238151, rs7312055, rs2158029, rs16941667, rs16941669)	ACAGCT	AA (26)	1.0 (ref)
	ATGGCT	AA (11)	**0.5 (0.3–0.8)**
CYP2E1 (rs915908, rs7092584, rs743535, rs2249695)	GCCC	EA (65)	1.0 (ref)
	GCCT	EA (10)	0.7 (0.6–0.9)
GPX2 (rs11623705, rs2412065, rs2737844)	GGC	EA (70)	1.0 (ref)
	GCT	EA (9)	0.7 (0.5–0.9)
SOD1 (rs4998557, rs10432782, rs2070424, rs1041740)	GTAC	EA (58)	1.0 (ref)
	AGGC	EA (6)	1.4 (1.1–1.9)
	GTAC	AA (52)	1.0 (ref)
	AGGC	AA (6)	0.6 (0.4–0.9)

[a]Criterion for selecting haplotypes for this table: ORs were statistically significant, or nearly so, after Bonferroni correction for multiple testing (13 for EA, 12 for AA)

[b]AA, African-American (black), EA, European-American (Caucasian/white).

[c]Unconditional logistic regression models for estimating main effect of each haplotype were adjusted for matching variables sex and age category and their 2-way interaction, and for continuous smoking duration rounded to whole years. The referent group for each OR was the most common haplotype. ORs highlighted in BOLD are statistically significant after Bonferroni correction of *P*-value.

Only one previous study examined effects on SCCHN incidence of any SNPs in oxidative stress pathways (26); it reported that rs2758346 in *SOD2* (which we did not study) was not associated with SCCHN.

We found no evidence of interaction with alcohol consumption for any oxidative stress SNP.

Genetic effects by race

Three SNPs in *SOD1* that had inverse effects in the 2 races were part of the *SOD1* haplotype that was also associated with differential effects by race. The direction of effect for carrying the minor allele of each individual SNP was consistent with the haplotype effect. The same is true for the 2 SNPs in *ADH1B* and *ADH4* that seemed to have different effects in African- and European-Americans.

Conclusions

CHANCE is one of the largest studies of head and neck cancer conducted in both African- and European-Americans. This study examined genetic polymorphisms in genes in the alcohol metabolism and oxidative stress biological pathways and estimated main effects of these polymorphisms along with their interaction with alcohol.

We selected tag SNPs to capture most of the variation in the 12 genes studied, rather than studying only missense SNPs within coding regions. However, an inherent limitation of genotyping common tag SNPs is that the method is likely to miss rare variants.

Because of small numbers of African-Americans compared with European-Americans, we could not definitively evaluate differences in SNP effects between races. We also had insufficient power to detect haplotype-drinking interaction because haplotypes were constructed and analyzed separately for African- and European-Americans. Small numbers overall also precluded precise estimation of interaction between SNPs and alcohol for allele frequencies less than 30%, or in relation to anatomic subsite.

Our study confirms findings of previous studies that the effects of many polymorphisms in alcohol metabolism pathways are modified by alcohol intake. However, most genetic variants in *ALDH2* and *CYP2E1* have been understudied and warrant additional investigation in light of the new associations that we report.

Our analysis of tag SNPs in *GPX2*, *SOD1*, and *SOD2* has identified several that are associated with SCCHN, hypopharyngeal, and oral cavity tumors. Confirmation of these findings in diverse populations is warranted.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Table 5. Additive interactive effects of alcohol with selected SNPs[a]

Gene, SNP, major/minor alleles Lifetime ethanol, mL	Homozygous for major allele: No. of cases/controls	Homozygous for major allele: Adjusted OR (95% CI)[b]	One or two copies of minor allele: No. of cases/controls	One or two copies of minor allele: Adjusted OR (95% CI)[b]	ICR[c] ($CI_{Bonferroni\ corrected}$)
ALDH2, rs2238151, T/C					
Never-drinkers	50/95	1.0 (ref)	67/184	**0.7 (0.4–1.1)**	**1.9 (0.1–3.8)**
>0 to <134,699	75/173	0.5 (0.3–0.9)	133/293	0.6 (0.4–1.0)	
134,699 to <757,550	97/138	0.8 (0.5–1.3)	220/222	1.2 (0.8–1.9)	
757,550+	122/65	**1.7 (1.0–2.8)**	381/106	**3.3 (2.0–5.3)**	
ADH1B, rs1159918, G/T					
Never-drinkers	49/101	1.0 (ref)	68/179	**0.9 (0.6-1.5)**	1.0 (-0.9 to 3.0)
>0 to <134,699	80/168	0.7 (0.4-1.1)	129/298	0.7 (0.5-1.1)	
134,699 to <757,550	107/129	1.1 (0.7-1.8)	211/231	1.3 (0.8-2.0)	
757,550+	140/58	**2.4 (1.4–4.1)**	365/115	**3.3 (2.1-5.4)**	
ADH7, rs1154460, G/A					
Never-drinkers	35/65	1.0 (ref)	81/215	**0.6 (0.4-1.1)**	0.9 (-0.6 to 2.4)
>0 to <134,699	57/131	0.5 (0.3-0.9)	152/334	0.6 (0.3-0.9)	
134,699 to <757,550	88/111	0.8 (0.5-1.4)	229/249	1.0 (0.6-1.6)	
757,550+	133/54	**1.9 (1.1-3.5)**	371/119	**2.5 (1.5-4.2)**	
CYP2E1, rs2249695, C/T					
Never-drinkers	73/136	1.0 (ref)	44/144	**0.6 (0.4–0.9)**	1.2 (-0.6 to 3.0)
>0 to <134,699	127/253	0.6 (0.4–0.9)	82/213	0.5 (0.3–0.8)	
134,699 to <757,550	160/178	1.1 (0.7–1.6)	158/181	1.0 (0.6–1.4)	
757,550+	218/92	**2.1 (1.4–3.3)**	286/81	**2.9 (1.8–4.6)**	

[a]Selected SNPs meet two criteria. They have (1) ICR confidence interval that either does not include 0 or nearly so, after Bonferroni correction, and (2) genotype information on sufficient numbers of cases and controls (at least 10 each) for calculating each of the 3 ORs highlighted in bold for that SNP. If ICR CI seemed significant but numbers of cases and controls were too sparse, SNP was judged to have insufficient evidence of interaction with alcohol, and was not included in this table.

[b]ORs for each SNP*drinking category were calculated from conditional logistic regression models including one SNP coded for dominant genetic model, categorized lifetime ethanol consumption, conditioned on sex, race, and age category, and adjusted for continuous smoking duration rounded to whole years. ORs highlighted in bold were used to calculate the ICR.

[c]ICRs that are statistically significant after Bonferroni correction are highlighted in bold. Bonferroni correction for 64 statistical tests.

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