The *ADH1C* Polymorphism Modifies the Risk of Squamous Cell Carcinoma of the Head and Neck Associated with Alcohol and Tobacco Use

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Abstract

Alcohol consumption interacts with tobacco use to increase the risk of head and neck squamous cell carcinoma (HNSCC). Alcohol is eliminated through oxidation by alcohol dehydrogenase (ADH). The *ADH1C* gene is polymorphic and the *ADH1C*1* allele metabolizes ethanol to acetaldehyde at a higher rate than the variant *ADH1C*2* allele. This polymorphism has been reported to alter the risk of HNSCC associated with alcohol use, although the literature differs in the estimates of both the magnitude and direction of this effect modification. We have investigated the association between the established risk factors for HNSCC and variant genotypes of *ADH1C* in a case-control study in the greater Boston area. *ADH1C* genotypes were determined from 521 cases and 599 population-based controls. The odds ratio (OR) for HNSCC associated with >26 drinks per week was 3.7 [95% confidence interval (95% CI), 2.4-5.7], whereas the OR for smoking >58 pack-years was 5.6 (95% CI, 3.8-8.4). The combination of heavy smoking and heavy drinking significantly interacted to produce an OR of 17.3 (95% CI, 7.8-38.3). In cases and controls, respectively, 16% and 14% were *ADH1C*1-1*, 46% and 46% were *ADH1C*1-2* and 38% and 40% were *ADH1C*2-2*. There was a significant interaction of alcohol use and genotype (P = 0.05), with an estimated oral cancer risk in heavy drinkers of 7.1 (95% CI, 2.3-22.0) for homozygous variants compared with an OR of 2.3 (95% CI, 1.4-3.8) for *ADH1C* homozygous wild type or heterozygous individuals (controlling for smoking, age, race, and gender). These findings suggest that the *ADH1C*2-2* genotype is associated with susceptibility to smoking and drinking-related HNSCC by modifying the biologically effective dose of alcohol. (Cancer Epidemiol Biomarkers Prev 2005;14(2):476–82)

Introduction

Head and neck squamous cell carcinoma (HNSCC) is the 10th most common cancer in the United States. Worldwide, HNSCC is the sixth most common cancer for both sexes and the third most common cancer in developing nations (1). The use of tobacco and alcohol accounts for ~75% of all HNSCCs in the United States (2, 3). Rothman (4) and Blot et al. (5) have shown that alcohol and tobacco have both independent and synergistic roles in the genesis of HNSCC. There is in fact, a strong dose-response relationship for both alcohol and tobacco consumption in virtually all of the HNSCC studies published to date. In addition, the well-established synergy between tobacco and alcohol suggests that much of alcohol's carcinogenic action involves the enhancement of the well-known carcinogenic effect of tobacco.

Whereas alcohol has not been found to cause cancer in animals (6), epidemiologic studies have shown that, among nonsmokers, heavy consumption of alcohol significantly increases an individual's risk of HNSCC (4, 7-9). However, the mechanism whereby alcohol induces HNSCC remains unclear. It has been postulated that acetaldehyde (the major intermediate metabolite of alcohol), which is a recognized carcinogen in animal models, may increase HNSCC cancer development in humans (10). Alternatively, ethanol may exert a deleterious effect on the cell directly, enhancing the uptake or activation of tobacco carcinogens (11). Consistent with this, ethanol has been shown to enhance the penetration of tobacco carcinogens across the oral mucosa (12).

There are well-known interindividual differences in alcohol metabolism (13). The majority of ethanol is eliminated in the liver via enzymatic oxidation to acetaldehyde and acetate, catalyzed by the various isoenzymes of alcohol dehydrogenase (ADH). ADH is a cytosolic, dimeric, zinc-containing NAD-dependent enzyme. This enzyme is a member of a large superfamily of medium-chain dehydrogenase/reductases able to catalyze the oxidation of a wide variety of endogenous and exogenous alcohols (13, 14). The oxidation of ethanol to acetaldehyde is the rate-limiting step in alcohol metabolism.

Alcohol dehydrogenase is composed of five well-described subunits (α, β, γ, π, and χ; ref. 15) encoded by seven genes, *ADH1* to *ADH7*, respectively. The class I ADH comprises *ADH1A*, *ADH1B*, and *ADH1C* (formerly called *ADH1*, *ADH2*, and *ADH3*), which are very closely related, being ~94% identical at the cDNA level. *ADH1B* and *ADH1C* are polymorphic with the metabolic rate for ethanol metabolism differing by allele (16, 17), such that the *ADH1C*1* allele has been shown to metabolize alcohol ~2.5 times faster than the *ADH1C*2* allele (18, 19). *ADH1C*1-1* homozygous individuals have the highest ethanol metabolism in the stomach and liver, whereas heterozygous (*ADH1C*1-2*) individuals have an intermediate and *ADH1C*2-2* homozygotes have the lowest level of alcohol metabolism (19). The *ADH1C* polymorphism has been shown to have an effect on the predisposition to alcoholism among Asian individuals, although this could be due to variation in the linked gene *ADH1B* (20).

Coutelle et al. (21) initially reported that French alcoholics with the *ADH1C*1-1* (previously called *ADH3*) genotype had an almost 4-fold greater risk of HNSCC than individuals without this genotype [95% confidence interval (95% CI),

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0.7-10.0]. In a case-control study of oral cancer in Puerto Rico, Harty et al. (22) reported a significantly increased cancer risk per additional alcoholic drink per week among persons with the *ADH1C*1-1* genotype. Among those individuals with the *ADH1C*1-1* genotype, individuals who consumed very high levels of alcohol (>56 drinks per week) had a 5.3-fold (95% CI, 1.0-28.8) increased risk of oral and pharyngeal cancer compared with *ADH1C*1-2* and *ADH1C*2-2* individuals. The reported interaction was more pronounced for the oral cavity than for the pharynx. However, Bouchardy et al. (23) did not confirm this observation in a subsequent case-control study of Caucasians in France.

Recent studies conducted in the United States have also yielded conflicting results. In a large population-based case-control study, Schwartz et al. (24) observed that among subjects reporting >29 alcoholic drinks per week, the *ADH1C*2-2* homozygous genotype was associated with a large, albeit imprecise odds ratio (OR) of 10.0 (95% CI, 2.5-40.2). In contrast, the risk among subjects with the same alcohol consumption but the *ADH1C*1-1* genotype was 6.1 (95% CI, 1.9-19.5). Olshan et al. (25) found no association among *ADH1C* genotype, alcohol consumption, and oral cancer. Finally, a recent pooled analysis of ADH genotypes and head and neck cancer observed an elevation of HNSCC risk associated with the *ADH1C*1-1* genotype (26). This Human Genome Epidemiology Review pooled data from six previously published *ADH1C* publications and included an international population of over 1,300 cases and 1,700 controls, with two of the studies contributing the bulk of the risk elevation.

In this study, we investigated the association of the *ADH1C* genotype in healthy population-based controls and in subjects with HNSCC. We examined the extent to which these genotypes modify cancer risk associated with exposure to alcohol and tobacco. Because it has been well established that alcohol and tobacco interact multiplicatively in causing HNSCC, we explicitly assessed how the *ADH1C* polymorphisms modify interaction of alcohol and tobacco on HNSCC risk.

Materials and Methods

We conducted a case-control study in Massachusetts from December 1999 to December 2003. The cases and controls were drawn from the Greater Boston Metropolitan Area. This region includes a population of ~3.5 million people in 249 cities and towns within a 1-hour drive of Boston. The institutional review boards at all participating institutions approved this study, and all volunteer participants provided informed consent.

Cases. Between December 1, 1999 and December 1, 2003, incident cases of HNSCC were identified through the multidisciplinary head and neck clinics, otolaryngology, and radiation oncology departments at nine medical facilities located in Boston, MA. The participating institutions included Beth Israel Deaconess Medical Center, Boston Medical Center, Boston Veterans Administration, Brigham and Women's Hospital, Dana-Farber Cancer Institute, Harvard Vanguard Medical Associates, Massachusetts Eye and Ear Infirmary, Massachusetts General Hospital and the New England Medical Center. According to the Massachusetts Cancer Registry (2000 data), the cases identified from these hospitals were generally comparable (with respect to age, gender and race) to all cases diagnosed in the Greater Boston Metropolitan area.

Eligibility requirements included being at least 18 years old, a resident in the study population, first diagnosis of HNSCC within the past 6 months, and being alive at the time of initial contact. We defined HNSCC as including *International Classification of Disease Codes, Ninth Revision* (ICD-9) codes 141, 143-6, 148, 149, and 161. All patients with carcinoma *in situ*, lip, salivary gland, or nasopharyngeal cancer or recurrent cancer of the head and neck region were excluded. Histologic classification of malignancy was based upon that reported by pathology at the participating hospitals. Cases were further classified by oral cavity (ICD-9 codes 140, 141, 143, 144, 145), pharynx (ICD-9 codes 146, 148 and 149), and larynx (ICD-9 code 161) to facilitate analysis of disease by site.

Controls. Population-based controls were drawn from the specified greater Boston population. The controls were frequency matched (1:1) to cases by age (±3 years), gender, and town of residence. These controls were identified through random selection from the Resident Lists for the 249 cities and towns within the study area. These annually compiled Resident Lists are mandated by state law and include all residents >17 years. The lists are believed to be 90% complete, with possible underlisting of illegal immigrants and the lowest income individuals. The resident lists include name, gender, year of birth, and usually occupation and last address (27).

Potential controls were randomly selected from the Resident List for a specific town using the cases' address as the starting point. Residents are arranged in the book by street address and precincts. A potential control of the same gender and age as the case is sought from this list, alternating the search direction through the book starting at the case address. Their address and telephone numbers are retrieved and the potential controls were invited to participate through an introductory letter. Individuals without telephone numbers were sent letters requesting them to contact the research staff. Subsequently the control was contacted by a telephone call to request participation and obtain consent (up to a maximum of 30 calls were made before the potential control was replaced). Controls were offered a US $40 incentive to participate.

Exposure Assessment. Participating cases and controls were given a self-administered questionnaire to collect medical history, demographic information, as well as information on tobacco and alcohol consumption. Each questionnaire was reviewed with each participant by a trained research coordinator. Smoking history was ascertained with a standardized instrument that assesses the number of years smoked, the number of cigarettes smoked per day, age at which an individual started smoking, number of years since quitting, and the duration of smoking. Similar information was obtained regarding lifetime consumption of beer, wine, and liquor. Questionnaires were given to case participants during an initial clinical visit and subsequently retrieved in person. Control participants received their questionnaires in the mail and returned them in person to the research assistant.

Venous blood samples were obtained from cases and controls. If a subject was unable or not willing to provide blood, a sample of buccal cells was obtained (a total of 187 subjects provided a buccal sample). Samples were stored at −80°C until DNA extraction was done by standard protocols.

Genotyping. Laboratory analyses were conducted by laboratory personnel who were blinded to case-control status. DNA was obtained either from whole blood or from exfoliated buccal cells. *ADH1C* genotyping was carried out by PCR amplification. Positive and negative controls were included in each batch of samples. Blood-derived DNA from individuals of known genotype (including all possible genotypes) served as a positive control whereas additional controls for detection of contamination etc. were routinely included in each genotype run. All control samples were concordant.

ADH1C genotype was determined by a modification of the method described by Harty et al. (22). A 145-bp section of the *ADH1C* gene was amplified by PCR with primers described by

Groppi et al. (28). The reaction product was then treated with *Ssp*I restriction enzyme. Samples were incubated overnight and then run on a Metaphor gel, stained with ethidium bromide.

Statistical Analysis. Tests for Hardy-Weinberg equilibrium among all controls were conducted. Exposures were classified as follows: pack-years of smoking were calculated as the product of current or former daily cigarette use (packs/d) and the duration of cigarette smoking (years). Nonsmokers were defined as subjects who had smoked zero pack-years or <100 cigarettes in their lifetime. The categorical levels for pack-years of tobacco consumption were determined by the quartile of use among all smokers. Light smokers were defined as those who consumed >0 to 35 pack-years, whereas heavy smokers were those who smoked >35 pack-years, the median among all smoking subjects. The number of alcohol drinks consumed per week was derived from the questionnaire for average lifetime weekly beer, wine, and liquor consumption. Nondrinkers were those individuals who consumed zero drinks of alcohol per week. The categorical levels for drinks per week were determined by the averages of total weekly consumption among all alcohol-consuming subjects. Light alcohol consumption was >0 up to 30 drinks per week, whereas heavy alcohol consumption was defined as >30 drinks per week of alcohol. The distributions of alcohol consumption and cigarette use were compared between the cases and controls and among genotype levels. ORs were calculated to measure the independent and synergistic effects of tobacco, alcohol, and genotype on the risk of HNSCC. Multiplicative interaction was assessed with two models: tobacco and alcohol (1), and alcohol and *ADH1C* (2). Variables for the joint effects were coded using a common referent group, and interactions were evaluated by estimating ORs for combinations of *ADH1C* genotype and exposure categories (alcohol and tobacco consumption). Unconditional logistic regression was done in SAS version 8 (SAS Institute, Cary, NC) to calculate the ORs and 95% CIs for the association between each of the interaction terms and cancer risk whereas controlling for age, gender, and race (29). Multivariate models included the matching terms age (continuous) and gender, as well as race. Frequency matching allows the use of unconditional regression with inclusion of the matching variables in the model (30). To test for the significance of the observed interactions, we conducted likelihood ratio tests using a χ^2 distribution.

Results

During the 4-year enrollment, 739 eligible patients with HNSCC were identified and 57 of these patients refused participation. Of the remaining 682, 107 either died or dropped out of the study before completion. Five hundred and seventy-five cases successfully completed the questionnaire and provided biological specimens. Of these participants, complete *ADH1C* and questionnaire data were available on 521 (71%). Similarly, 1,447 potential controls were identified and invited by telephone to participate in the study and 630 (44% response rate) agreed to participate, with three subsequently choosing to cease their involvement in the study. Of these participants, *ADH1C* genotype and questionnaire data were available on 599 (41%). The characteristics of the final study population are described in Table 1.

Among the 521 cases and 599 controls the mean age was 60 and 61 years, respectively. Twenty-eight percent of cases and 28% of controls were female. Among controls, 90% reported their race to be Caucasian compared with 86% of the cases. The overall mean weekly alcohol consumption among all cases was 26 drinks per week, whereas controls reported a mean of 12 alcoholic drinks per week. The median level of alcohol intake reported among controls that were drinkers was eight drinks per week compared with 18 drinks per week for cases that were drinkers. Cases were more likely to consume >30 drinks of alcohol (heavy drinking) per week than controls (27% versus 9%). Heavy alcohol consumption was associated with an almost 4-fold increased risk for HNSCC compared with nondrinkers, adjusted for age, race, and gender (OR, 3.8; 95% CI, 2.5-6.0). The mean pack-years of smoking among cases was 38 pack-years, compared with 21 among controls. The median reported cigarette use in controls among smokers was 27 pack-years compared with 43 pack-years in cases. In addition, cases were more likely to have heavy (≥35 pack-years) cigarette consumption than controls (48% versus 26%). Similarly, heavy tobacco use was associated with a >5-fold elevation in HNSCC risk, adjusted for age, race, and gender (OR, 3.7; 95% CI, 2.6-5.1).

The prevalence of *ADH1C* genotypes among cases and controls is shown in Table 1. The *ADH1C* genotypes were in Hardy-Weinberg equilibrium among all controls (P = 0.3), limiting this calculation to whites did not alter the result. The prevalence of *ADH1C*1-1* was 38% among cases and 40% among controls, *ADH1C*1-2* was 46% among cases and 46% among controls, and the prevalence of *ADH1C*2-2* was 16% among cases and 14% among controls. The multivariate adjusted risks of HNSCC are shown by *ADH1C* genotype in Table 1. Table 1 also shows the ORs for tobacco and alcohol exposure by cancer site (oral cavity, pharynx, and larynx). The magnitude of these risks generally parallels that for all sites, with the exception of suggestion of a more marked elevation of risk for laryngeal cancer associated with smoking. The *ADH1C* polymorphism was not independently associated with risk for HNSCC. After adjustment for age, gender, race, alcohol, and tobacco use, risk among individuals with the *ADH1C*2-2* genotype was not significantly higher than among those with the *ADH1C*1-1* genotype (OR, 1.2; 95% CI, 0.9-1.8). The *ADH1C*1-1* and *ADH1C*1-2* genotypes were combined in subsequent analyses for purposes of power and stability of estimation.

As expected, the combination of both smoking and drinking increased an individual's risk for developing HNSCC (Table 2). Nonsmoking individuals who consumed >26 alcohol drinks per week had a 2-fold elevated risk for HNSCC, adjusted for age and gender, compare to those who did not drink (OR, 1.8; 95% CI, 1.1-3.1). Similarly, nondrinking individuals with a history of >58 pack-years of cigarette consumption had over a 3-fold greater risk for HNSCC, compared with nonsmokers (OR, 3.5; 95% CI, 2.2-5.5). When both tobacco and alcohol were evaluated jointly, consumption of >26 drinks of alcohol per week and >58 pack-years of cigarette use was associated with an imprecise yet significant 17-fold increased risk of HNSCC (OR, 17.3; 95% CI, 7.8-38.3) Interestingly, we consistently observed that consumption of less than six drinks (but greater than zero) of alcohol per week was associated with a decreased (albeit nonsignificant) risk of HNSCC, relative to nondrinkers. When we compared the interaction of heavy smoking and heavy alcohol use by cancer site, the OR for oral cavity was 15.8 (P for interaction = 0.004), the OR for pharynx was 21.3 (P for interaction = 0.048), and the OR for laryngeal cancer was 24.2 (P for interaction = 0.6).

The interaction between *ADH1C* and alcohol intake and HNSCC risk are shown in Table 3. Although *ADH1C* genotype was not independently associated with an elevated risk for HNSCC, a fairly imprecise 7-fold increase in risk was observed among *ADH1C*2-2* individuals who consumed at least 30 drinks per week of alcohol (OR, 7.1; 95% CI, 2.3-22.0). In contrast, heavy drinkers with the combined *ADH1C*1-1* and *ADH1C*1-2* genotype had only a 2-fold increased risk for cancer (OR, 2.3; 95% CI, 1.4-3.8) and the test for interaction was significant (P = 0.05). Again, we observed that among the light alcohol drinkers (regardless of genotype) there was a diminished risk for cancer, relative to nondrinkers and *ADH1C* genotype did not alter the magnitude of the HNSCC risk

among the light drinkers. The differential effect of *ADH1C* was observed only among heavy drinkers. When these data were stratified by cancer site, a significant interaction between heavy alcohol use and *ADH1C* genotype was evident only for oral cancer ($P = 0.03$).

Figure 1 shows the association of *ADH1C* with the predicted risk of HNSCC for increasing alcohol consumption. The dose-response by genotype diverges beginning at ~15 drinks per week, becoming most pronounced at very high alcohol use levels.

Although our *a priori* hypothesis was that the *ADH1C* alleles would modify the carcinogenic risk associated with alcohol, we also examined HNSCC risk with the interaction of *ADH1C* genotype and tobacco. Individuals with the *ADH1C*2-2* genotype who were heavy smokers had 4-fold elevated cancer risk (OR, 4.2; 95% CI, 2.2-8.1). In contrast, individuals with the *ADH1C*1-1* or *ADH1C*1-2* genotype who were heavy smokers only had a 3-fold elevated cancer risk (OR, 2.7; 95% CI, 1.8-3.8) with a significant interaction ($P = 05$).

Discussion

As expected, we observed a significant interaction between heavy alcohol consumption and cigarette smoking resulting in an elevated HNSCC risk. In evaluating the extent to which the *ADH1C* polymorphism modifies the HNSCC risk, we observed a significant interaction between the *ADH1C* variant genotype and alcohol consumption such that the *ADH1C*2-2* genotype conferred a large, significant elevation in HNSCC risk among the heavier alcohol drinkers (≥30 drinks/wk). In a similar analysis of heavy smoking, we found that *ADH1C* variant genotype to have a less dramatic effect on HNSCC risk, suggesting that the homozygous variant genotype of the *ADH1C* gene acts by altering the effective ''dose'' of alcohol.

Several prior studies have suggested that the more ''rapid'' alcohol metabolizing allele of the *ADH1C* gene (the *ADH1C*1* allele) is positively associated with oral cancer risk or plays no role in modifying HNSCC risk (21, 23, 25, 31). Our study, along with that of Schwartz et al. (24), suggests that the opposite may be true. Schwartz et al. reported that *ADH1C*2-2* individuals who consumed >29 drinks per week have a 10-fold increase in HNSCC risk compared with a 6-fold enhanced risk among the *1-1* individuals. Similar to our work, they did not observe this effect modification among subjects consuming <29 alcoholic drinks per week.

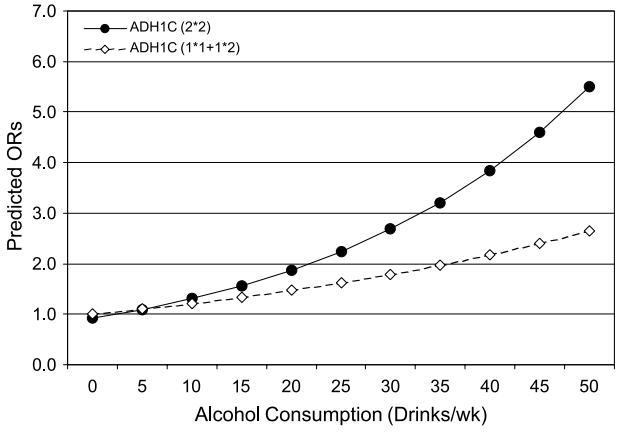

Figure 1. Relationship between the predicted ORs for HNSCC and the interaction of alcohol consumption with *ADH1C* genotype. Regression model adjusted for age, gender race, and tobacco use.

Harty et al. (22) reported that the risk of oral cancer was higher among subjects with the *ADH1C*1-1* genotype than among the group of individuals with the combined *ADH1C*1-2/ADH1C*2-2* genotype, (OR, 1.3; 95% CI, 0.8-2.4). In addition, they found, among individuals who consumed at least 57 drinks per week, that the adjusted risk for oral cancer was 5.3 times (95% CI = 1.0-28.8) higher among persons with the *ADH1C*1-1* genotype than among the combined group of subjects with the *ADH1C*1-2* or *ADH1C*2-2* genotype.

One critical difference to note among the studies is the striking variation among the prevalence of cases defined as ''heavy'' alcohol drinkers. In our study, 27% cases and 9% of the controls consumed >30 drinks per week. Olshan et al. (25) observed 23% of the cases and 3% of the controls consumed ≥60 drinks per week. The study by Schwartz et al. (24) reported the lowest prevalence among cases, 17% of cases and 4% of controls reported alcohol consumption ≥43 drinks per week. In contrast, Harty et al. (22) reported the highest proportion of heavy consumers, where 46% of cases and 9% of controls consumed >57 alcoholic drinks per week. If the gene-environment interaction these investigators have similarly studied is mechanistically driven by nonlinear kinetics, detection and assessment of the magnitude of any interaction might differ between studies because of the variation in alcohol use of the populations.

In attempting to understand the discrepancy observed among the different studies of *ADH1C* genotype and HNSCC, it is important to note that the investigations have been conducted in different ethnic groups. Harty et al. (22) studied Puerto Ricans, Bouchardy et al. (23), and Coutelle et al. (21) studied French Caucasians; Zavras et al. (31) investigated oral cancer risk in residents of Greece. Schwartz and Olshan conducted their work in Washington state and North Carolina, respectively. Our study enrolled subjects from the greater Boston area. Accordingly, the observed discrepancy in interaction (or lack of interaction) between the *ADH1C* polymorphism and alcohol consumption with the risk of HNSCC may be ethnic variability and population stratification associated with different genetic linkage among different ethnic groups, as illustrated by Nelson et al. (32) in their study of the prevalence of the glutathione *S*-transferase theta deletion polymorphism (showing geographic differences in polymorphism prevalence within Caucasians).

The *ADH* gene cluster is located on chromosome 4q. There are six known loci (*ADH1-ADH6*) that map together at 4q21-25. The *ADH1B* and *ADH1C* loci have been observed to be polymorphic (15, 33). The known polymorphisms exhibit varying degrees of linkage (20, 34). There also is now substantial evidence that suggests particular variant forms of the *ADH* genes greatly influence ethanol metabolism and, thereby, may also influence ethanol consumption (16, 18, 20, 35, 36). Similar to *ADH1C 1*, the *ADH1B*2* allele more rapidly metabolizes ethanol to acetaldehyde (17, 37). The prevalence of the *ADH1B* and *ADH1C* polymorphisms differ dramatically by race and ethnicity. Among Asians, the *ADH1B 2* and *ADH1C 1* are the more common alleles (38), although among Caucasians, the prevalence of *ADH1B 1* is ~90% and the *ADH1C 2* is equally distributed with *ADH1C 1* (39).

In Asians, the allele frequencies of *ADH1B 2* and *ADH1C 1* are significantly decreased in alcoholics compared with the general population (40-43). These studies have repeatedly shown a greater risk of alcohol dependence associated with the presence of the *ADH1B 1* allele than with the *ADH1B 2* allele, even when controlling for the variation in the aldehyde dehydrogenase activity that is also prevalent in Asian populations. Studies that examined the association of the prevalence of the *ADH1C*1* allele with alcohol dependence show an inverse association among Asians between *ADH1C*1* and alcohol use. This difference has been shown to be

Table 1. Participant characteristics of HNSCC cases and controls

Characteristics	Cases, *n* (%)	Controls, *n* (%)	All HNSCC, OR (95% CI)*	Oral cavity only, OR (95% CI)†	Pharynx only, OR (95% CI)‡	Larynx only, OR (95% CI)§
Age (y)						
<52	143 (27.4)	141 (23.5)				
>52-60	133 (25.5)	153 (25.5)				
>60-68	123 (23.6)	151 (25.2)				
>68	122 (23.4)	154 (25.7)				
Gender						
Male	375 (72.0)	430 (71.8)				
Female	146 (28.0)	169 (28.2)				
Race						
Caucasian	446 (85.6)	540 (90.2)				
Black	23 (4.4)	21 (3.5)				
Other	50 (9.6)	37 (6.2)				
Missing	2 (0.4)	1 (0.2)				
Tobacco (pack-years)						
0	100 (19.2)	198 (33.1)	1.0	1.0	1.0	1.0
>0-15	77 (14.8)	129 (21.5)	1.2 (0.8-1.7)	1.0 (0.6-1.5)	1.5 (0.8-2.6)	1.9 (0.8-4.3)
>15-35	92 (17.7)	118 (19.7)	1.7 (1.2-2.5)	1.8 (1.2-2.8)	1.5 (0.8-2.7)	2.5 (1.1-5.6)
>35-58	107 (20.5)	94 (15.7)	2.6 (1.8-3.7)	2.0 (1.3-3.3)	2.7 (1.5-4.9)	5.6 (2.6-12)
>58	145 (27.8)	60 (10.0)	5.6 (3.8-8.4)	4.0 (2.5-6.6)	5.6 (3.1-10)	14.8 (6.9-32)
Tobacco (pack-years)‖						
None	100 (19.2)	198 (33.1)	1.0	1.0	1.0	1.0
Light	169 (32.4)	247 (41.2)	1.4 (1.0-1.9)	1.3 (0.9-2.0)	1.5 (0.9-2.5)	2.1 (1.0-4.4)
Heavy	252 (48.4)	154 (25.7)	3.7 (2.6-5.1)	2.7 (1.8-4.1)	3.9 (2.3-6.5)	8.9 (4.4-18)
Alcohol (drinks/wk)						
0	81 (15.5)	108 (18.0)	1.0	1.0	1.0	1.0
>0-6	90 (17.3)	190 (31.7)	0.7 (0.5-1.0)	0.6 (0.4-1.0)	0.7 (0.4-1.3)	0.9 (0.5-1.0)
>6-11	74 (14.2)	113 (18.9)	1.0 (0.7-1.5)	1.0 (0.6-1.7)	1.0 (0.5-2.1)	1.2 (0.7-1.5)
>11-26	119 (22.8)	117 (19.5)	1.7 (1.1-2.5)	1.5 (0.9-2.5)	1.9 (1.0-3.5)	2.2 (1.1-2.5)
>26	156 (29.9)	69 (11.5)	3.7 (2.4-5.7)	4.0 (2.3-6.7)	4.5 (2.4-8.4)	2.9 (2.4-5.7)
Missing	1 (0.2)	2 (0.3)				
Alcohol (drinks/wk)¶						
None	81 (15.5)	108 (18.0)	1.0	1.0	1.0	1.0
Light	298 (57.2)	433 (72.3)	1.0 (0.7-1.4)	0.9 (0.6-1.4)	1.1 (0.6-1.8)	1.4 (0.7-2.6)
Heavy	141 (27.1)	56 (9.3)	3.8 (2.5-6.0)	4.2 (2.5-7.2)	4.9 (2.6-9.3)	2.6 (1.2-5.8)
Missing	1 (0.2)	2 (0.3)				
ADH1C						
1*1	197 (37.8)	240 (40.1)	1.0	1.0	1.0	1.0
1*2	242 (46.4)	273 (45.6)	1.1 (0.9-1.4)	1.2 (0.9-1.7)	0.8(0.6-1.2)	1.3 (0.8-2.1)
2*2	82 (15.7)	86 (14.4)	1.2 (0.9-1.8)	1.1 (0.7-1.7)	1.0 (0.6-1.8)	1.9 (1.0-3.5)
Total	521	599				

*All HNSCC (521 cases), adjusted for age, gender, and race.
†Oral cavity (256 cases, includes ICD-9 codes 140, 141 143, 144, and 145), adjusted for age, gender, and race.
‡Pharynx (149 cases, includes ICD-9 codes 146, 148, and 149), adjusted for age, gender, and race.
§Larynx (106 cases, includes ICD-9 code 161), adjusted for age, gender, and race.
‖Cutpoint for heavy tobacco is 35 pack-years.
¶Cutpoint for heavy alcohol is 30 per week.

predominately between the *ADH1C*1-1* subjects compared with the heterozygous or homozygous *ADH1C*2* individuals (17). It has recently been suggested that most of the genetic variation in alcohol dependence that is not attributable to aldehyde dehydrogenase activity is related primarily to the *ADH1B* polymorphic allele, and the linkage between the functional variants at *ADH1B* and the functional variants at the *ADH1C* locus has generated an apparent spurious association of alcohol ingestion and the *ADH1C* polymorphism (20, 42, 43). In studies that did not consider this linkage, the *ADH1C 2* allele has been associated with higher alcohol intake and alcoholism (18). Differences in alcohol use and dependence among Caucasians have also been reported and attributed primarily to *ADH1B* genetic variants (17), despite the fact that the prevalence of these variants is ethnically quite different (20, 22, 36, 39). Little is known about possible ethnic and racial variation in the prevalence of this polymorphism, and the linkage of this variant with the *ADH1C* and *ADH1B* polymorphisms has also not been described.

Previous authors (21, 22, 26) have suggested that the action of alcohol in oral carcinogenesis is mediated through its metabolite, acetaldehyde. Whereas oral mucosal ADH may contribute to local acetaldehyde production, the oral microbiota are responsible for higher levels of production. Homann et al. (44) observed that smoking and heavy drinking were the strongest predictors of increasing microbial acetaldehyde production. In addition, they suggest that the oral flora produces the majority of oral and salivary acetaldehyde. Therefore, interindividual variation in ADH seems less likely to alter HNSCC risk via an alteration of acetaldehyde production. We propose that the action of alcohol is direct rather than through a metabolic intermediate, perhaps enhancing the carcinogenic effect of tobacco. Figure 1 supports this suggestion; because the *2*2* genotype is associated with a 2.5-fold slower alcohol metabolism, the variant ADH is perhaps giving rise to a higher biologically effective dose of alcohol. Our observation of a smaller interaction between ADH and tobacco also suggests that ADH exerts its major effects via modification of the alcohol dose. Larger studies are needed to further analyze potentially meaningful three way and four way interactions.

Discrepancies among these studies might also be affected by recall bias or selection bias. It has been shown that cancer cases, especially those with greater alcohol intake, tend to underestimate their alcohol consumption to a greater degree than controls (45, 46). Underestimation of actual alcohol consumption reported by cases in our study may diminish

Table 2. Interaction of tobacco and alcohol on HNSCC risk ORs and 95% CIs

Terms	Tobacco (pack-years)*				
	0	>0-15	>15-35	>35-58	>58
Alcohol (drinks/wk)*					
0	1.0	1.2 (0.8-1.7)	1.4 (1.0-2.1)	1.7 (1.1-2.6)	3.5 (2.2-5.5)
>0-6	0.7 (0.5-1.0)	0.8 (0.5-1.3)	1.0 (0.6-1.7)	1.1 (0.6-2.0)	2.3 (1.3-4.2)
>6-11	0.9 (0.6-1.4)	1.1 (0.6-1.9)	1.3 (0.8-2.4)	1.6 (0.9-2.9)	3.2 (1.8-5.9)
>11-26	1.4 (0.9-2.1)	1.6 (0.9-2.8)	2.0 (1.2-3.4)	2.4 (1.4-4.2)	4.9 (2.7-8.8)
>26	1.8 (1.1-3.1)	2.1 (1.1-4.0)	2.6 (1.4-4.8)	6.5 (3.2-13.0)	17.3 (7.8-38.3)

NOTE: Adjusted for age, gender, and race.
*Categories determined by quartiles among all exposed individuals.

the magnitude of the association between alcohol and HNSCC, resulting in attenuated power to detect effect modification. Whereas every attempt has been made to administer the questionnaire in an identical fashion to both cases and controls, there still remains the possibility a differential misclassification may arise through normal variability among interviewers. However, these biases are not likely to segregate by genotype and hence are less likely to affect the noted association of gene-environment interaction.

Whereas the role of selection bias in our study should be diminished through the use of population-based controls, only 575 of 739 (78%) of all eligible cases and 44% of eligible controls fully participated. Fifty-six (8%) of the 682 consented cases died and another 51 cases (8%) ceased participation before collection of the questionnaire or blood. It is possible that the cases that died before completion may represent more advanced cases with a heavier burden of alcohol and tobacco exposure. This may partially be responsible for the lower prevalence of heavy smokers and drinkers we observe compared with other published studies. In addition, cases that sought care at participating institutions may be different than all cases from the study area. Enrolled cases may be more educated and of higher income than cases that refused to participate. Therefore, it is possible that the selection of cases and controls was influenced by unmeasured factors related to socioeconomic status. Any selection bias in our study could lead to the cases and controls being more similar with respect to their tobacco and alcohol use. Thus, the effects of tobacco and alcohol may be diminished, which in turn could lead to a weakening of any possible gene-environment interaction.

Our results suggest that the *ADH1C* allele modifies the carcinogenic dose-response for alcohol and tobacco in HNSCC, giving rise to a significant gene-environment interaction consistent with data from the largest study previously conducted in the United States (24). We further suggest that alcohol has a direct effect on the oral mucosa and that cancer risk is not mediated by its metabolites. Further investigation must be undertaken with sample sizes sufficiently large to detect meaningful associations between additional genotypes, alcohol, tobacco, dietary factors and cancer risk. In addition, it will be critical to include large numbers of ethnically diverse individuals to assess the precise role of linkage of loci in genetic interaction among alcohol metabolism, tobacco use, and HNSCC risk.

Table 3. Interaction of *ADH1C* genotype and alcohol on HNSCC risk

Alcohol*	*ADH1C* (1*1 + 1*2)			*ADH1C* (2*2)		
	Cases	Controls	OR (95% CI)†	Cases	Controls	OR (95% CI)†
None	69	87	1.0	12	21	0.8 (0.4-1.8)
Light	255	372	0.9 (0.6-1.3)	43	61	0.9 (0.6-1.6)
Heavy	114	52	2.3 (1.4-3.8)	27	4	7.1 (2.3-22.0)

*Cutpoint for heavy alcohol is >30 drinks/wk test for heavy × ADH interaction ($P = 0.05$).
†Adjusted for age, gender, race, and smoking.

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