Short Communication

Alcohol Dehydrogenase 3 Genotype Is Not Associated with Risk of Squamous Cell Carcinoma of the Oral Cavity and Pharynx

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Abstract

Alcohol is one of the major risk factors for oral and pharyngeal cancer. The rate-limiting step in alcohol metabolism is the oxidation (activation) of ethanol to acetaldehyde by the alcohol dehydrogenases (ADHs). It has been hypothesized that individuals who are homozygous for the fast allele (ADH31–1) are at greater risk for alcohol-related cancers. To test this hypothesis, we investigated the association between the ADH3 genotype and oral and pharyngeal cancer risk in a large racially homogeneous case-control study of 229 patients and 575 matched control subjects with frequency matching on age, sex, and smoking status. Although the smoking status was matched between cases and controls, current and former alcohol use remained a significant risk factor, compared with never use (odds ratio, 2.08; 95% confidence interval, 1.37–3.17; odds ratio, 1.97; 95% confidence interval, 1.25–3.09; and odds ratio, 1.00, respectively). The ADH31 allele frequency of controls was 57.4%, consistent with reports of similar racial groups (50–60%). The genotype distribution in controls was also consistent with the Hardy-Weinberg equilibrium (P = 0.51). However, the ADH31 allele frequency and ADH31–1 genotype frequency were not significantly different between cases and controls [55.5% versus 57.4% (P = 0.52), and 30.6% versus 31.3% (P = 0.91), respectively]. There was no association between ADH3 genotypes (ADH31–1, ADH31–2, and ADH31–1) and risk of oral and pharyngeal cancer (odds ratios, 1.00; 0.96; 95% confidence interval, 0.68–1.37; and odds ratio, 1.23; confidence interval, 0.78–1.93, respectively). Therefore, we found no evidence that a main effect of ADH3 genotype or a combined effect of alcohol and ADH1 genotype on risk of cancer of the oral cavity or pharynx.

Introduction

Alcohol is one of the major risk factors for oral and pharyngeal cancer, although pure ethanol has never been shown to be carcinogenic in animal experiments. The rate-limiting step in alcohol metabolism is the oxidation (activation) of ethanol to acetaldehyde by the ADHs.3 The ADH type 3 gene (ADH3) exists as an ADH31 wild-type allele and as an ADH32 polymorphic allele in Caucasian populations. Enzymes encoded by the ADH32 allele activate ethanol twice as fast as those encoded by the ADH31 allele (1). Therefore, it has been hypothesized that individuals who are homozygous for the fast allele (ADH31–1) are at greater risk for alcohol-related cancers. To date, three studies have tested this hypothesis with mixed results (2–4), and additional studies have been recommended to clarify the role of ADH31–1 (2, 4, 5). Consequently, we attempted to independently replicate the association between the ADH31–1 genotype and oral and pharyngeal cancer risk in a larger racially homogeneous case-control study with frequency matching on age, sex, and smoking status.

Materials and Methods

Subjects. The details of subject recruitment were described previously (6). Briefly, from May 1995 until April 2000, patients with newly diagnosed and histologically confirmed squamous cell carcinoma of the oral cavity and pharynx (excluding cancers of the nasopharynx, lip, or salivary glands) were recruited at our institution. Cancer-free control subjects were recruited from the largest multispecialty managed care organization in the Houston metropolitan area (7) and were frequency-matched to case subjects on age, sex, and smoking status derived from questionnaires. These healthy controls did not have benign or neoplastic conditions and were not participants of the cancer-screening clinics. After informed consent, each subject donated 30 ml of blood and completed a questionnaire. For this genotyping study, there were 35 cases who donated blood samples after treatment. Once the subjects were contacted, the participation rate was 90% among the cases and 73.3% among the controls. The study protocol was approved by the M. D. Anderson and Kelsey Seybold Institutional Review Boards.

Genotyping. Genotyping for ADH3 was performed on DNA extracted from peripheral blood lymphocytes as described previously (Fig. 1; Ref. 3). To eliminate the possibility of racial confounding, only Caucasians (non-Hispanic whites) were included in this study.

3 The abbreviation used is: ADH, alcohol dehydrogenase.
Statistical Analysis. The distributions of matching variables, including age, sex, ethnicity (only Caucasians), and smoking status, were first examined for the adequacy of the matching procedure. In the multivariate logistic regression analysis, dummy variables of the genotypes were created to calculate odds ratios and 95% confidence intervals with further adjustment for age, sex, and smoking status in addition to alcohol use. All of the statistical tests were two-sided and were performed with Statistical Analysis System software (Version 6; SAS Institute Inc., Cary, NC).

Results
Genotyping for the ADH3 polymorphism was performed on 236 case subjects with oral and pharyngeal cancer and on 613 control subjects without cancer. Genotyping was unsuccessful on 7 case subjects and 8 control subjects, and alcohol data were unavailable on 30 control subjects. Consequently, the analysis included 229 patients and 575 matched control subjects (Table 1). The larger number of control subjects was included to provide a stable estimate of genotype frequencies. None of the subgroups listed in Table 1 had any significant differences in the presence of at least one ADH3-1 allele and ADH3-2-1 genotype frequency compared with other subgroups (data not shown).

The presence of at least one ADH3-1 allele in the control group was 57.4%, consistent with reports of similar racial groups (50–60%; Refs. 1, 2, 4, 8, 9). The genotype distribution in the controls was consistent with the Hardy-Weinberg equilibrium (P = 0.51), suggesting no influence from selection of control subjects. The presence of at least one ADH3-1 allele and ADH3-2-1 genotype frequency were not significantly different between case subjects and control subjects [55.5% versus 57.4% (P = 0.52), and 30.6% versus 31.3% (P = 0.91), respectively]. Also, the presence of at least one ADH3-1 allele and ADH3-2-1 genotype frequency were similar in patients with primaries of the oral cavity and patients with primaries of the pharynx (55.8% versus 55.1%, and 30.6% versus 30.6%, respectively). There was no association between ADH3 genotypes (ADH3-1, ADH3-2, and ADH3-2-1) and risk of oral and pharyngeal cancer (odds ratios, 1.00; 0.96; 95% confidence interval, 0.68–1.37; and odds ratio, 1.23; 95% confidence interval, 0.78–1.93, respectively; Table 2). However, both current and former alcohol use were significantly associated with risk of oral and pharyngeal cancer, compared with never use (odds ratio, 2.08; 95% confidence interval, 1.37–3.17; odds ratio, 1.97; 95% confidence interval, 1.25–3.09; and odds ratio, 1.00, respectively; Table 2) even after controlling for age, sex, and smoking status.

Discussion
Although previous studies (2–4) have reported a trend of increased risk of oral and pharyngeal cancer for individuals possessing the ADH3-1 genotype, our findings do not support this hypothesis. Recently, several authors have raised questions concerning the reporting of genetic associations of diseases that are not simple Mendelian single-gene disorders (10–13). Some authors suggested guidelines for such genetic association studies including biological plausibility, significant power, proper matching and selection of controls, and, most importantly, independent replication (10).

Biological plausibility is the basis from which these studies should arise. It is plausible that the presence of at least one ADH3-1 allele would put one at a greater risk of cancer by creating higher concentrations of acetaldehyde. Because, the ADH3-2 allele has been linked to a greater risk of alcoholism (14), it also could be associated with a greater risk of oral and pharyngeal cancer. Thus, it is also plausible that the presence of at least one ADH3-1 allele has no significant impact on risk or that the slow allele (ADH3-2) puts one at a greater risk. In fact, we found that among alcohol (both former and current) users, the ADH3-2 allele was more frequent in case subjects than in control subjects (Table 2). Another alcohol metabolic enzyme aldehyde dehydrogenase 2 encoded by ALDH2 is also polymorphic in Japanese subjects but not in Caucasians and found to be associated with excessive alcohol consumption in Japanese men (15). However, the findings of a recent case-control study did not support the role of genetic polymorphisms of ALDH2 in risk of oral cancer among Japanese subjects (16).

Secondly, study size is crucial to obtaining reliable estimates of allele frequencies and for performing meaningful subgroup analyses. This study included over 800 subjects, which is 30% larger than all of the previous reports combined (2–4). The first report of ADH3 genotype and the risk of pharyngeal cancer contained only 21 cases and 37 control subjects (2), and the genotype distribution in control subjects nearly departed from the Hardy-Weinberg equilibrium (P = 0.08). The other studies (3, 4) performed subgroup analyses with referent categories containing fewer than seven case subjects. The study by Harty et al. (3), the main finding of which was the association between heavy alcohol use and the ADH3-1 genotype, used a referent category of only 5 case subjects and 15 control subjects, and a majority of cells had

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**Table 1**: Subject characteristics and smoking status

<table>
<thead>
<tr>
<th></th>
<th>Case subjects (n = 229)</th>
<th>Control subjects (n = 575)</th>
<th>P (^{\alpha} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤49</td>
<td>54 (23.6)</td>
<td>136 (23.7)</td>
<td>0.212</td>
</tr>
<tr>
<td>50–59</td>
<td>50 (21.8)</td>
<td>149 (25.9)</td>
<td></td>
</tr>
<tr>
<td>59–69</td>
<td>68 (29.7)</td>
<td>183 (31.8)</td>
<td></td>
</tr>
<tr>
<td>&gt;69</td>
<td>57 (24.9)</td>
<td>107 (18.6)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td>0.273</td>
</tr>
<tr>
<td>Male</td>
<td>145 (63.3)</td>
<td>340 (59.1)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>84 (36.7)</td>
<td>235 (40.9)</td>
<td></td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td>0.586</td>
</tr>
<tr>
<td>Non-Hispanic whites</td>
<td>229 (100.0)</td>
<td>575 (100.0)</td>
<td></td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>65 (28.4)</td>
<td>159 (27.7)</td>
<td></td>
</tr>
<tr>
<td>Former</td>
<td>101 (44.1)</td>
<td>237 (41.2)</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>63 (27.5)</td>
<td>179 (31.1)</td>
<td></td>
</tr>
</tbody>
</table>

\( ^{\alpha} \chi^2 \).
fewer than 10 subjects. Consequently, in seeming contradiction, the odds ratio for the $ADH_3^{1-1}$ genotype was significantly elevated compared with the $ADH_3^{1-2}$ or $ADH_3^{2-2}$ genotypes for the heaviest drinkers (>56 drinks/week), whereas the odds ratio for the $ADH_3^{2-2}$ genotype was elevated significantly compared with the $ADH_3^{1-2}$ or $ADH_3^{1-1}$ genotypes for the mid-level drinkers (15–56 drinks/week). Such inconsistencies in risk estimates point out the problems of sample size and suggest caution in the interpretation of these subgroup analyses.

Finally, proper matching and control selection are critical in genotyping studies where population admixture may influence the genotype frequencies and, thus, the ultimate results. The study of Coutelle et al. (2) included only male alcoholics, and there was a 10-year difference in average age between case subjects and control subjects. Bouchardy et al. (4) used hospital control subjects who suffered from various diseases and made no attempt to match for smoking. In the study of Harty et al. (3), case subjects and control subjects were not matched on age ($P = 0.002$), sex ($P = 0.006$), or smoking status (no information was provided), and less than 50% of eligible subjects participated. Such population admixture may result in spurious associations, particularly if a study includes genetically distinct subpopulations (13) or a racially heterogeneous group (3). All of these discrepancies represent possible selection biases that could impact results.

Although our study is the largest to the best of our knowledge, our study lacks the specifics (drinks/week) of alcohol intake, which makes it difficult to perform subgroup analyses and to precisely evaluate the combined effect of alcohol and $ADH_3$ genotype as other studies did. However, we did find that alcohol use remained a significant risk factor for oral and pharyngeal cancer despite the cases and controls being matched on smoking status, but we found no evidence that supports a main effect of $ADH_3$ genotype or a combined effect of alcohol and $ADH_3$ genotype on risk of cancer of the oral cavity or pharynx in our study population.

However, it is likely that the crude assessment of alcohol consumption in this study and use of nonpopulation-based controls may have caused misclassification bias. Larger studies with more precise assessments for alcohol consumption and population-based controls are needed to verify our findings.

References


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