Original Article

**Prognostic Value of Drinking Status and Aldehyde Dehydrogenase 2 Polymorphism in Patients With Head and Neck Squamous Cell Carcinoma**

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Received December 2, 2014; accepted August 31, 2015; released online January 23, 2016

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**ABSTRACT**

**Background:** The association between alcohol drinking, aldehyde dehydrogenase 2 (ALDH2) polymorphism, and survival in patients with head and neck squamous cell carcinoma (HNSCC) remains unclear.

**Methods:** We performed a retrospective cohort study of 267 HNSCC patients at Aichi Cancer Center. Of these, 65 patients (24%) were non-drinkers, 104 (39%) were light drinkers (ethanol <46 g or <5 days/week), 46 (17%) were moderate drinkers (ethanol intake 46–68 g/day and ≥5 days/week), and 52 (20%) were heavy drinkers (ethanol intake ≥69 g and ≥5 days/week). The prognostic value of pre-treatment drinking status and ALDH2 polymorphism was investigated using multivariate proportional hazard models.

**Results:** Drinking status was associated with disease-free survival (DFS) in HNSCC patients, with marginal statistical significance (5-year DFS: 67.9% [95% confidence interval {CI}, 53.8–78.4%] for non-drinkers, 57.6% [95% CI, 47.4–66.6%] for light drinkers, 46.1% [95% CI, 30.8–60.1%] for moderate drinkers, and 43.5% [95% CI, 29.3–56.9%] for heavy drinkers; \( P = 0.088 \)). However, this association lost significance when multivariate analyses were adjusted for established prognostic factors. ALDH2 genotype was not significantly associated with DFS in HNSCC patients (5-year DFS: 85.7% [95% CI, 53.9–96.2%] for Lys/Lys, 56.2% [95% CI, 47.4–64.1%] for Glu/Lys, and 50.5% [95% CI, 40.3–59.7%] for Glu/Glu; \( P = 0.154 \)). After stratification by ALDH2 genotype, we observed a significant positive dose-response relationship between drinking status and DFS in HNSCC patients with ALDH2 Glu/Glu (\( P_{\text{trend}} = 0.029 \)).

**Conclusions:** In this study, we identified a significant positive dose-response relationship between pre-treatment drinking status and DFS in HNSCC patients with ALDH2 Glu/Glu. To confirm this association, further study is warranted.

**Key words:** alcohol drinking; ALDH2; head and neck cancer; squamous cell carcinoma; survival

**INTRODUCTION**

Worldwide, almost 600 000 new cases of head and neck cancer are reported each year.¹ Alcohol drinking is an established risk factor for head and neck squamous cell carcinoma (HNSCC).²³ In general, ethanol is oxidized by alcohol dehydrogenase (ADH) enzymes to acetaldehyde, which is then further oxidized to acetate by aldehyde-dehydrogenase (ALDH) enzymes. This latter oxidation is largely dependent on the ALDH2 enzyme.⁴ The metabolism of ethanol leads to accumulation of acetaldehyde, which is toxic and established as a strong carcinogen, and differences in ethanol metabolism that result from polymorphisms in the genes that code for these enzyme affect cancer etiology among drinkers.⁵ The ALDH2 Glu504Lys polymorphism (Single Nucleotide Polymorphism Database [dbSNP] ID, rs671), which has a catalytically
inactive subunit, is known to interact with the association between alcohol drinking and HNSCC risk.\textsuperscript{5–13} Light drinkers with $ALDH2$ Lys/Lys and Glu/Lys have 18 times and 5 times higher average peaks of acetaldehyde concentrations in blood, respectively, than moderate drinkers with $ALDH2$ Glu/Glu.\textsuperscript{14} However, the prognostic value of alcohol drinking and $ALDH2$ polymorphism on clinical outcome in patients with HNSCC remains unclear.\textsuperscript{15–21}

Previously, we clarified the association between lifestyle factors and prognosis in HNSCC patients.\textsuperscript{22} Alcohol drinking is known to induce factors associated with cancer survival.\textsuperscript{23–25} This suggests that alcohol drinking might affect the survival of HNSCC patients and that $ALDH2$ polymorphism might interact with this association.

Here, we conducted a retrospective cohort study to clarify the potential association of these factors among 267 patients treated for HNSCC at Aichi Cancer Center (ACC).

**METHODS**

**Patients**

We selected patients from the database of the Hospital-based Epidemiologic Research Program at Aichi Cancer Center (HERPACC), based at ACC in Nagoya, Japan. The HERPACC framework has been detailed elsewhere.\textsuperscript{22} Our questionnaire consisted of items related to smoking and drinking habits in the period preceding the development of the present symptoms or reason for visit to ACC. Levels of alcohol consumption were divided into four groups: non-, light, moderate, and heavy drinkers. Moderate drinkers were defined as individuals who consumed alcoholic beverages in a daily amount of $\geq 46$ g ethanol (equivalent to two Japanese drinks) but $<69$ g ethanol for $\geq 5$ days per week. Heavy drinkers were defined as individuals who consumed alcohol beverages in a daily amount of $\geq 69$ g ethanol for $\geq 5$ days per week. Light drinkers were defined as individuals who consumed alcoholic beverages in a daily amount of $<46$ g ethanol for $<5$ days per week. The remaining patients were categorized as non- or light drinkers using data from the HERPACC study for head and neck cancers.\textsuperscript{29}

Cumulative exposure to cigarette smoking was quantified as pack-years of smoking (PY), the product of the number of packs consumed per day and number of years of cigarette smoking. We divided patients into four groups based upon PY: non-, light (PY < 20), moderate (PY $\geq 20 – 39$), and heavy smokers (PY $\geq 40$).

**Statistical methods**

The primary endpoint of this study was disease-free survival (DFS), which was defined as the number of days from the beginning of treatment to the date of relapse. The associations between drinking status (0: non, 1: light, 2: moderate, and 3: heavy), $ALDH2$ genotypes (0: Glu/Lys, 1: Glu/Glu, and 2: Lys/Lys), and DFS were evaluated by the Kaplan-Meier product-limit method and uni- and multivariate Cox proportional hazards models. Confounders considered in the multivariate analyses were age (continuous), sex (male or female), ECOG PS (0–2), smoking status (non- vs light vs moderate vs heavy), Union for International Cancer Control (UICC) stage (1–4), and treatment method (surgery or CRT/RT).
Median age was 61 (range, 21–77) years, and median follow-up time was 5.0 years (range, 0.7 months–9.1 years). Drinking was more prevalent among males than females. Similarly, drinking was more prevalent among smokers, but less prevalent among oral cavity cancer patients than patients without oral cavity cancer. Five-year DFS among all patients was 55.5% (95% confidence interval [CI], 49.1–61.4%).

**Impact of drinking status and ALDH2 genotype on DFS**

Figure 1 shows the Kaplan-Meier survival curves for drinking status. Drinking status was associated with DFS in HNSCC patients with marginal statistical significance (5-year DFS: 67.9% [95% CI, 53.8–78.4%] for non-drinkers, 57.6% [95% CI, 47.4–66.6%] for light drinkers, 46.1% [95% CI, 30.8–60.1%] for moderate drinkers, and 43.5% [95% CI, 29.3–56.9%] for heavy drinkers; \( P = 0.088 \)).

Figure 2 shows the Kaplan-Meier survival curves for ALDH2 genotype. ALDH2 genotype was not significantly associated with DFS in HNSCC patients (5-year DFS: 85.7% [95% CI, 53.9–96.2%] for Lys/Lys, 56.2% [95% CI, 47.4–64.1%] for Glu/Lys, and 50.5% [95% CI, 40.3–59.7%] for Glu/Glu; \( P = 0.154 \)).

Table 2 shows the results of univariate and multivariate analysis for DFS. In univariate analysis, drinking status showed a significant trend in decreasing DFS (\( P_{\text{trend}} = 0.013 \)). However, neither drinking status nor ALDH2 genotype showed a significant association with DFS in multivariate analysis.

**Interaction between drinking status and ALDH2 genotype on DFS**

Kaplan-Meier survival curves of DFS for drinking status according to ALDH2 genotype are shown in Figure 3. Drinking status was significantly associated with DFS in ALDH2 Glu/Glu patients (59.4% [95% CI, 30.9–79.4%] for...
non-drinkers, 60.6% [95% CI, 44.8–73.2%] for light drinkers, 44.6% [95% CI, 23.5–63.8%] for moderate drinkers, and 21.1% [95% CI, 5.8–42.7%] for heavy drinkers; \( P = 0.023 \).

In multivariate analysis, drinkers with \( \text{ALDH2 Glu/Glu} \) showed a significant trend toward decreased DFS (\( P_{\text{trend}} = 0.029; \) Table 3). In contrast, among patients with \( \text{ALDH2 Glu/Lys} \), drinking status showed no significant association with DFS. In addition, we observed suggestive heterogeneity between drinking status and \( \text{ALDH2} \) genotype on DFS (\( P \) for heterogeneity = 0.100).

**DISCUSSION**

In this study, we demonstrated that high pre-treatment alcohol consumption worsens DFS in HNSCC patients. This effect was evident only among the \( \text{ALDH2 Glu/Glu} \) patients, suggesting that it might differ by \( \text{ALDH2} \) genotype. To our knowledge, this is the first report to evaluate the impact of alcohol drinking combined with \( \text{ALDH2} \) genotype on clinical outcome in HNSCC patients.
Although the mechanism behind this association between drinking status and survival of HNSCC patients remains unclear, several explanations appear plausible based on existing evidence. First, alcohol drinking may induce activation of NF-κB, a transcription factor that has been linked with the transformation of cells and survival of cancer stem cells.\textsuperscript{30} Additionally, NF-κB regulates the expression of genes associated with apoptosis, proliferation, invasion, and other biological processes.

### Table 2. Impact of drinking status and ALDH2 genotype on disease-free survival in patients with head and neck squamous cell carcinoma

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>n (relapse)</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=n</td>
<td>HR (95% CI)</td>
<td>P-value</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td>Drinking status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non</td>
<td>65</td>
<td>1.00</td>
<td>reference</td>
<td>—</td>
</tr>
<tr>
<td>Light</td>
<td>104</td>
<td>1.38</td>
<td>0.83–2.29</td>
<td>0.210</td>
</tr>
<tr>
<td>Moderate</td>
<td>46</td>
<td>1.80</td>
<td>1.02–3.18</td>
<td>0.042</td>
</tr>
<tr>
<td>Heavy</td>
<td>52</td>
<td>1.90</td>
<td>1.09–3.31</td>
<td>0.023</td>
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<td></td>
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<tr>
<td>ALDH2 genotype (rs671)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glu/Glu</td>
<td>111</td>
<td>1.00</td>
<td>reference</td>
<td>—</td>
</tr>
<tr>
<td>Glu/Lys</td>
<td>142</td>
<td>0.98</td>
<td>0.68–1.40</td>
<td>0.914</td>
</tr>
<tr>
<td>Lys/Lys</td>
<td>14</td>
<td>0.34</td>
<td>0.11–1.08</td>
<td>0.067</td>
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<td></td>
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</tr>
</tbody>
</table>

ALDH2, aldehyde dehydrogenase 2; CI, confidence interval; HR, hazard ratio.

Drinking status was classified as follows: non, light (<46 g ethanol or <5 days/week), moderate (46–68 g ethanol for ≥5 days/week), and heavy (≥69 g ethanol for ≥5 days/week).

Adjusted for age, sex, performance status, clinical disease stage, primary tumor site, smoking, and definitive treatment.

Figure 3. Kaplan Meier survival curves of disease-free survival for drinking status according to aldehyde dehydrogenase 2 (ALDH2) Glu/Glu genotype. Five-year disease-free survival among ALDH2 Glu/Lys patients was 63.6% (95% confidence interval [CI], 43.4–78.2%) for non-drinkers, 55.1% (95% CI, 40.8–67.3%) for light drinkers, 47.1% (95% CI, 25.2–66.3%) for moderate drinkers, and 58.6% (95% CI, 37.3–72.3%) for heavy drinkers (logrank test, P = 0.764). Respective rates among ALDH2 Glu/Glu patients were 59.4% (95% CI, 44.8–73.2%), 44.6% (95% CI, 23.5–63.8%), and 21.1% (95% CI, 5.8–42.7%) (logrank test, P = 0.023).

Although the mechanism behind this association between drinking status and survival of HNSCC patients remains unclear, several explanations appear plausible based on existing evidence.
angiogenesis, and metastasis of cancer. Second, alcohol consumption may induce TP53 mutation, which is associated with HNSCC survival. Third, alcohol drinking may modify the DNA methylation profile in HNSCC cells. DNA methylation modifications may affect the prognosis of HNSCC. Fourth, alcohol drinking damages normal mucosa in the head and neck region. Long-term continuation of this mucosal damage, called “field cancerization”, may be associated with a predisposition to relapse or development of a second primary tumor (SPT). However, the incidence of SPT was not associated with drinking status in this study (data not shown).

In addition, our findings suggest that the association between alcohol drinking and prognosis of HNSCC may differ by ALDH2 genotype. Although this mechanism is also unclear, we speculate that this association might be affected by alcohol dependence. Individuals who are heterozygous or homozygous for the Lys allele of ALDH2 Glu504Lys polymorphism (rs671) have greatly reduced ability to metabolize acetaldehyde, which greatly decreases their risk for alcohol dependence. After definitive treatment, ALDH2 Glu/Glu patients might maintain higher levels of alcohol consumption than ALDH2 Glu/Lys patients. However, this point should be assessed in other studies evaluating post-treatment or under-treatment drinking behavior according to ALDH2 genotype.

Our study has several methodological strengths. First, because clinicians involved in the care of study patients were not aware of the exposure status examined in this study, information bias was less likely to have been introduced. Second, because the analyses were adjusted for established prognostic factors, including clinical disease stage and PS, the observed associations were theoretically independent. However, the exclusion of residual confounding, including that due to HPV infection, cannot be fully excluded. Finally, the moderate sample size may have limited the ability of the study to detect differences between the groups.

### Table 3. Impact of drinking status according to ALDH2 genotype on disease-free survival in patients with head and neck squamous cell carcinoma

<table>
<thead>
<tr>
<th>ALDH2 genotype (rs671)</th>
<th>Drinking status</th>
<th>n</th>
<th>n (relapse)</th>
<th>Non</th>
<th>Light</th>
<th>Moderate</th>
<th>Heavy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glu/Glu</td>
<td>20</td>
<td>5</td>
<td>1.13</td>
<td>0.42–3.01</td>
<td>0.814</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>Glu/Lys</td>
<td>33</td>
<td>9</td>
<td>1.27</td>
<td>0.57–2.86</td>
<td>0.557</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Lys/Lys</td>
<td>12</td>
<td>2</td>
<td>2.00</td>
<td>0.22–10.04</td>
<td>0.535</td>
<td>2</td>
</tr>
</tbody>
</table>

Drinking status was classified as follows: non, light (<46 g ethanol or <5 days/week), moderate (46–68 g ethanol for ≥5 days/week), and heavy (≥69 g ethanol for ≥5 days/week).

Table 3. Impact of drinking status according to ALDH2 genotype on disease-free survival in patients with head and neck squamous cell carcinoma. ALDH2, aldehyde dehydrogenase 2; HR, hazard ratio; CI, confidence interval.
We concluded that high pre-treatment alcohol drinking worsened DFS in patients with HNSCC. This effect might be clearer among patients with ALDH2 Glu/Glu. Our results suggest a possible gene-environmental interaction in the clinical outcome of HNSCC patients. Replication in a larger study is warranted.

ONLINE ONLY MATERIAL

Abstract in Japanese.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the energy and contribution of the doctors, nurses, technical staff, and hospital administration staff at Aichi Cancer Center Hospital for their daily management of the HERPACC study. This study was supported by a Grant-in-Aid for Scientific Research on Innovative Areas (No. 221S0001) and Kakenhi (26253041) from the Ministry of Education, Culture, Sports, Science and Technology of Japan, Grants-in-Aid for Cancer Research from the Ministry of Health, Labour and Welfare of Japan, and the Japan Society for the Promotion of Science A3 Foresight Program.

Conflicts of interest: None declared.

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