Research Article

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The Influence of Prediagnosis Alcohol Consumption and the Polymorphisms of Ethanol-Metabolizing Genes on the Survival of Head and Neck Cancer Patients

Wei-Ting Lee[1,2], Jenn-Ren Hsiao[1,2], Chun-Yen Ou[1], Cheng-Chih Huang[1], Chan-Chi Chang[1,2], Sen-Tien Tsai[1], Ken-Chung Chen[3], Jehn-Shyun Huang[3], Tung-Yiu Wong[3], Yu-Hsuan Lai[2,4], Yuan-Hua Wu[4], Wei-Ting Hsueh[4], Shang-Yin Wu[2,5], Chia-Jui Yen[2,5], Jang-Yang Chang[5,6], Chen-Lin Lin[7], Ya-Ling Weng[6], Han-Chien Yang[6], Yu-Shan Chen[1], and Jeffrey S. Chang[6]

Abstract

Background: Although alcohol drinking is an established risk factor of head and neck cancer (HNC), less is known about its role in the prognosis of HNC. The current study investigated the association between pretreatment alcohol consumption and the overall survival (OS) of HNC patients.

Methods: Cox proportional hazards models were performed to evaluate the association between prediagnosis alcohol drinking and the OS of HNC patients. In addition, the influence of the polymorphisms of two ethanol-metabolizing genes, *ADH1B* and *ALDH2*, on this relationship was also evaluated.

Results: The results showed a significant positive dose–response relationship between prediagnosis alcohol use and worse OS of HNC patients. This association was more significant for oropharyngeal cancer, hypopharyngeal cancer, and laryngeal cancer than for oral cancer. The association between alcohol use and the poorer OS of HNC patients was mainly through its association with a higher stage of HNC at diagnosis. The worst OS associated with alcohol use was observed among HNC patients with the fast *ADH1B* and the slow/nonfunctional *ALDH2* genotype combination.

Conclusions: Our analysis showed a significant positive dose–response relationship between prediagnosis alcohol use and a worse OS of HNC. This association was mainly due to the higher stage of HNC among alcohol drinkers. In addition, the polymorphisms of the ethanol-metabolizing genes, *ADH1B* and *ALDH2*, modified the relationship between prediagnosis alcohol use and the OS of HNC patients.

Impact: Prediagnosis alcohol use may be a prognostic indicator of HNC.

Introduction

Each year, approximately 600,000 new cases of head and neck cancer (HNC; cancers of the oral cavity, oropharynx, hypopharynx, and larynx) are diagnosed worldwide (1). Most of the HNCs can be attributed to the use of alcohol, betel quid, and cigarette (2). Recently, the incidence of human papillomavirus–associated oropharyngeal cancer has been increasing (3). Unlike the well-established role of lifestyle factors in the development of HNC, less is known about the prognostic lifestyle factors of HNC. Although the 5-year survival of HNC patients has increased in the past few decades from 53% in 1982 to 1986 to 66% in 2002 to 2006 (4), there is still room for improvement. Investigating the role of lifestyle factors in the prognosis of HNC may provide additional insights for improving the outcomes of HNC.

Alcohol drinking is a major risk factor of HNC, accounting for approximately 39% of the HNCs in Western countries (5). In Taiwan, approximately 22% of the HNCs could be attributed to alcohol use (6). Less is known regarding the influence of alcohol on the prognosis of HNC. Ten published studies have evaluated the relationship between alcohol use and HNC outcomes with inconsistent results (7–16). Among the ten studies, three observed no significant association between prediagnosis alcohol use and HNC survival (11, 14, 15). Although the other seven all found an association between alcohol use and a poorer HNC survival, the results were inconsistent with regards to the subsite of HNC and the level of alcohol consumption (7–10, 12, 13, 16).

The major carcinogen of alcohol is acetaldehyde, which is produced through the metabolism of ethanol by alcohol

[1]Department of Otolaryngology, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University, Tainan, Taiwan. [2]Institute of Clinical Medicine, College of Medicine, National Cheng Kung University, Tainan, Taiwan. [3]Department of Stomatology, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University, Tainan, Taiwan. [4]Department of Radiation Oncology, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University, Tainan, Taiwan. [5]Division of Hematology/Oncology, Department of Internal Medicine, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University, Tainan, Taiwan. [6]National Institute of Cancer Research, National Health Research Institutes, Tainan, Taiwan. [7]Department of Nursing, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University, Tainan, Taiwan.

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Corresponding Author: Jeffrey S. Chang, National Health Research Institutes, 1st F. 367 Shengli Road, Tainan 70456, Taiwan. Phone: 886-6-208-3422, ext. 65160; Fax: 886-6-208-3427; E-mail: jeffreychang@nhri.org.tw

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dehydrogenase (17). Acetaldehyde is then converted to the nontoxic acetate by aldehyde dehydrogenase (17). The SNPs of the ethanol-metabolizing genes (*ADH1B* rs1229984 and *ALDH2* rs671) can determine the speed at which acetaldehyde is generated and metabolized and influence an individual's susceptibility to the carcinogenicity of alcohol. To date, only two studies have considered the effect of ethanol-metabolizing genes on the association between alcohol drinking and HNC outcomes, and the results were inconsistent (11, 14).

The current study aimed to study the association between prediagnosis alcohol consumption and the overall survival (OS) of HNC patients. In addition, the interaction between the polymorphisms of *ADH1B* and *ALDH2* and prediagnosis alcohol drinking on the OS of HNC patients was also investigated.

Materials and Methods

This study was approved by the Institutional Review Boards of the National Health Research Institutes and the National Cheng Kung University Hospital. All participants of the study provided a written-informed consent. The current study was conducted in accordance with the Declaration of Helsinki.

Study subject recruitment

All of the HNC cases of this analysis were from an ongoing HNC case–control study that began subject recruitment on September 1, 2010, in the Department of Otolaryngology and the Department of Stomatology at the National Cheng Kung University Hospital. Eligible criteria included (1) diagnosis of pathologically confirmed squamous cell carcinoma of the head and neck, including cancers of the oral cavity, oropharynx, hypopharynx, and larynx (ICD-10 codes: C00-C10, C12-C14, C32); (2) no history of any previous cancer diagnosis; and (3) aged 20 years or older. For the current study, we included subjects recruited before February 25, 2016.

Data collection by interview

An in-person interview was conducted with each subject using a standardized questionnaire to ascertain data on alcohol consumption before HNC diagnosis. The interview on alcohol use began by asking each subject whether he/she ever drank alcohol. Further questions were administered to those with a positive response, including (1) starting age; (2) quitting age if the subject had quit drinking alcohol for more than 6 months prior to the diagnosis of HNC; (3) alcoholic beverage type (beer, wine, and liquor); and (4) drinking frequency (monthly, weekly, or daily) and the volume (in milliliters) consumed each time. Data on potential confounders, including sex, age, education, and consumption of betel quids and cigarettes, were also collected.

Clinical information and vital status

Clinical information, including cancer stage (American Joint Committee on Cancer Staging, Seventh Edition), treatment modality, and the vital status of the HNC patients were obtained from the cancer registry of the National Cheng Kung University Hospital. Clinical information in the hospital cancer registry is maintained and regularly updated by the oncology case manager according to the medical records. The vital status in the hospital tumor registry is regularly updated according to the vital status provided by the Health Promotion Administration of Taiwan, by the medical records, and by patient follow-up by the oncology case manager. The last date of the follow-up period for this analysis is July 12, 2017.

Blood sample collection

A pretreatment blood sample was collected from each subject in an EDTA-containing vacutainer tube. Buffy coat was obtained after the centrifugation of the blood sample. DNA was then extracted from the buffy coat using a commercially available DNA purification kit. DNA samples were kept in a −80°C refrigerator until ready to use.

Genotyping

Taqman-based allelic discrimination method on an Applied Biosystems 7500 Real-Time Polymerase Chain Reaction System (Applied Biosystems) was used for genotyping *ADH1B* rs1229984 and *ALDH2* rs671. Because DNA samples were unavailable for 23 subjects (3.2%), genotyping was performed with DNA samples of 717 (96.9%) subjects. Duplicate genotyping was performed for 10% of the samples for quality control purpose, and the results showed 100% concordance.

Statistical analysis

Cox proportional hazards models and Kaplan–Meier analyses were performed to evaluate the association between prediagnosis alcohol drinking and the OS of HNC patients. The total time of follow-up was calculated from the date of diagnosis to the date of death for individuals who died during the follow-up period or censored on July 12, 2017 (the last date of follow-up) for those that were alive at the end of the follow-up.

The alcohol variable was examined: (1) by drinking status: never + occasional drinker, former regular drinker, and current regular drinker. Regular drinking = drinking alcohol at least once per week; (2) by frequency: never, monthly, weekly, and daily; (3) by the level of drinking: nondrinker (0 g per day), light drinker (<14 g/day), moderate drinker (14–42 g/day), and heavy drinker (>42 g/day). The grams of alcohol per day were calculated according to alcoholic beverage type, volume, and frequency using the formula: total volume of alcohol per day × alcohol content × 0.798 g/mL. The alcohol content was set at 5% for beer, 13% for wine, and 40% for liquor, and 0.798 g/mL is the density of ethanol. The grams per day of alcohol from different beverage types were added together to obtain the total grams of alcohol per day; and (4) by drink-years with 1 drink-year = 1 drink (14 g of alcohol) per day × alcohol drinking for 1 year. The drink-years were then divided into four groups, including 0 drink-year for never drinkers and tertiles (0.1–54.9 drink-years, 55–159.9 drink-years, and 160 or more drink-years) for ever drinkers. Because the data on alcoholic beverage type were not collected before March 20, 2011, 73 subjects (9.9%) without data on alcoholic beverage type were excluded from the analysis with the level of alcohol drinking and drink-years.

Univariate Cox proportional hazards models were performed to assess the association between the OS of HNC patients and the covariates, including age, sex, education, use of betel quids and cigarettes, HNC stage, HNC grade, and treatments (surgery, radiotherapy, and chemotherapy). The covariates significantly ($P < 0.05$) associated with the OS of HNC patients were included in the multivariable Cox proportional hazards models. A directed acyclic graph (18) was used to assess the relationship between prediagnosis alcohol drinking, the covariates, and the OS of HNC patients.

Table 1. The association between demographic, lifestyle factors, and clinical characteristics and the OS of HNC patients

	***N* = 740**		
	***n* (%)**	**HR (95% CI)[a]**	***P***
Age at diagnosis, mean (SE)	55.19 (0.39)	1.00 (0.98–1.01)	0.68
Sex			
Male	693 (93.65)	Reference	
Female	47 (6.35)	0.64 (0.32–1.31)	0.22
Education			
≦Elementary school	204 (27.57)	Reference	
Junior high	217 (29.32)	0.76 (0.53–1.11)	0.15
High school/technical school	242 (32.70)	0.81 (0.56–1.16)	0.24
Some college or more	77 (10.41)	0.68 (0.39–1.19)	0.18
Cigarette smoking			
Never	108 (14.61)	Reference	
Former	139 (18.81)	1.17 (0.67–2.03)	0.58
Current	492 (66.58)	1.46 (0.92–2.32)	0.10
Betel quid chewing			
Never	222 (30.00)	Reference	
Former	280 (37.84)	1.00 (0.70–1.43)	1.00
Current	238 (32.16)	1.11 (0.77–1.59)	0.57
Stage			
1, 2	300 (40.54)	Reference	
3, 4	405 (54.73)	4.35 (2.95–6.41)	<0.0001
Unknown	35 (4.73)	2.57 (1.18–5.58)	0.02
Grade			
Low	229 (30.95)	Reference	
Moderate or high	429 (57.97)	1.73 (1.20–2.50)	0.004
Unknown	82 (11.08)	3.23 (2.05–5.09)	<0.0001
Surgery			
No	232 (31.35)	Reference	
Yes	495 (66.89)	0.35 (0.26–0.46)	<0.0001
Unknown	13 (1.76)	0.38 (0.09–1.54)	0.18
Radiotherapy			
No	352 (47.57)	Reference	
Yes	359 (48.51)	2.88 (2.08–3.97)	<0.0001
Unknown	29 (3.92)	1.47 (0.63–3.43)	0.37
Chemotherapy			
No	412 (55.68)	Reference	
Yes	304 (41.08)	3.53 (2.58–4.83)	<0.0001
Unknown	24 (3.24)	2.04 (0.88–4.72)	0.10
Treatment combination			
Radiotherapy or chemotherapy or both	189 (25.54)	Reference	
No treatment	25 (3.38)	0.72 (0.35–1.48)	0.37
Surgery only	310 (41.89)	0.19 (0.13–0.29)	<0.0001
Surgery + radiotherapy or chemotherapy	46 (6.22)	0.35 (0.18–0.70)	0.003
Surgery + radiotherapy + chemotherapy	138 (18.65)	0.64 (0.44–0.93)	0.02
Treatment combination uncertain[b]	32 (4.32)	0.41 (0.19–0.88)	0.02

Abbreviation: *N*, number.
[a]HR and 95% CI were calculated using Cox proportional hazards model.
[b]Information was unknown for one or more of the treatment modalities.

The association between prediagnosis alcohol drinking and the OS of HNC patients was evaluated by the different subsites of HNC (oral cavity, oro- and hypopharynx, and larynx) to evaluate whether the relationship of alcohol and the OS of HNC patients might differ by the subsites of HNC.

To evaluate the influence of ethanol-metabolizing genes on the association between prediagnosis alcohol consumption and the OS of HNC patients, Cox proportional hazards models were performed stratified by the genotypes of *ADH1B* and *ALDH2*.

Results

During the recruitment period (September 1, 2010–February 25, 2016), 974 eligible subjects were identified and 740 (76%) agreed to participate in the study. These 740 HNC cases included 467 oral cancers, 176 oropharyngeal + hypopharyngeal cancers, 86 laryngeal cancers, and 11 cancers of multiple HNC subsites. The HNC cases were all incident cases, with 97.4% interviewed within 1 month of the diagnosis, 1.9% interviewed between 1.0 and 1.9 months after the diagnosis, and 0.7% interviewed between 2.0 and 5.9 months after the diagnosis. The majority (93.6%) of the HNC patients were men (Table 1). Forty-three percent of the subjects had completed at least a high school education. Eighty-five percent and 70% of the subjects were ever cigarette smokers and ever betel quid chewers, respectively. More than half of the cases were diagnosed at stages 3 and 4 and had a histology of moderate or high grade. Sixty-seven percent of the patients underwent surgery, 49% received radiotherapy, and 41% received chemotherapy.

Among the 740 HNC patients, 186 (25.1%) died during the follow-up. The median follow-up time was 3.1 years. Age, sex, education, cigarette smoking, and betel quid chewing were not

Table 2. The association between prediagnosis alcohol use and the OS of HNC patients

Prediagnosis alcohol use	*n* (%)	Model 1 HR (95% CI)[a] unadjusted	*P*	Model 2 HR (95% CI)[a] adjusted for stage	*P*	Model 3 HR (95% CI)[a] adjusted for stage and grade	*P*	Model 4 HR (95% CI)[a] adjusted for stage, grade, and treatment	*P*
Never + occasional	239 (32.30)	Referent		Referent		Referent			
Former	99 (13.38)	1.78 (1.14–2.79)	0.01	1.53 (0.97–2.40)	0.07	1.54 (0.98–2.42)	0.06	1.54 (0.98–2.43)	0.06
Current	402 (54.32)	1.38 (0.98–1.94)	0.07	1.23 (0.87–1.74)	0.24	1.25 (0.89–1.77)	0.20	1.16 (0.82–1.65)	0.40
Former + current	501 (67.70)	1.45 (1.04–2.02)	0.03	1.29 (0.92–1.79)	0.13	1.31 (0.94–1.83)	0.12	1.24 (0.88–1.73)	0.22
Frequency									
Never	214 (28.92)	Referent		Referent		Referent			
Monthly	25 (3.38)	0.34 (0.08–1.39)	0.13	0.37 (0.09–1.52)	0.17	0.42 (0.10–1.75)	0.23	0.41 (0.10–1.72)	0.22
Weekly	75 (10.14)	1.21 (0.71–2.07)	0.48	1.14 (0.67–1.95)	0.63	1.14 (0.66–1.94)	0.64	1.00 (0.57–1.74)	1.00
Daily	408 (55.14)	1.41 (1.00–1.98)	0.05	1.24 (0.88–1.75)	0.22	1.28 (0.90–1.81)	0.17	1.21 (0.85–1.72)	0.29
Unknown	18 (2.43)	—	—	—	—				
		P trend = 0.03		*P* trend = 0.15		*P* trend = 0.11		*P* trend = 0.20	
Amount[b]									
Never	198 (29.69)	Referent		Referent		Referent		Referent	
Light	97 (14.54)	0.98 (0.57–1.68)	0.95	0.86 (0.50–1.47)	0.57	0.93 (0.54–1.59)	0.78	0.85 (0.49–1.47)	0.56
Moderate	95 (14.24)	1.43 (0.88–2.34)	0.15	1.17 (0.71–1.90)	0.54	1.12 (0.68–1.85)	0.66	1.13 (0.68–1.88)	0.64
Heavy	259 (38.83)	1.46 (0.99–2.14)	0.05	1.25 (0.85–1.84)	0.25	1.23 (0.83–1.82)	0.30	1.13 (0.76–1.68)	0.54
Unknown	18 (2.70)	—	—	—	—	—	—	—	—
		P trend = 0.03		*P* trend = 0.15		*P* trend = 0.22		*P* trend = 0.38	
Drink-years[c]									
0	198 (29.69)	Referent		Referent		Referent		Referent	
0.1–54.9	145 (21.74)	1.16 (0.74–1.82)	0.53	1.02 (0.65–1.61)	0.92	1.08 (0.68–1.70)	0.75	0.99 (0.62–1.57)	0.95
55–159.9	144 (21.59)	1.31 (0.84–2.05)	0.24	1.10 (0.70–1.72)	0.69	1.02 (0.64–1.61)	0.94	1.00 (0.63–1.60)	0.99
160 or more	146 (21.89)	1.63 (1.07–2.49)	0.02	1.38 (0.90–2.11)	0.14	1.37 (0.89–2.09)	0.15	1.23 (0.79–1.89)	0.36
Unknown	34 (5.10)	—							
		P trend = 0.02		*P* trend = 0.13		*P* trend = 0.19		*P* trend = 0.36	

Abbreviation: *N*, number.

[a]HR and 95% CI were calculated using Cox proportional hazards model.

[b]Light: < 14 g/day; moderate: 14–42 g/day; heavy: > 42 g/day.

[c]1 drink-year = 1 drink (14 g of alcohol) per day x 1 year.

significantly associated with the OS of HNC patients ($P > 0.05$; Table 1). Advanced stages (stages 3 and 4) were associated with a poorer OS compared with early stages [stages 1 and 2; HR = 4.35; 95% confidence interval (CI), 2.95–6.41; $P < 0.0001$]. Higher histologic grades were associated with a worse OS compared with low histologic grade (moderate grade: HR = 1.53; 95% CI, 1.04–2.25; $P = 0.03$; high grade: HR = 2.59; 95% CI, 1.62–4.14; $P < 0.0001$). Surgery was associated with a better OS (HR = 0.35; 95% CI, 0.26–0.46; $P < 0.0001$), whereas radiation (HR = 2.88; 95% CI, 2.08–3.97; $P < 0.0001$) and chemotherapy (HR = 3.53; 95% CI, 2.58–4.83; $P < 0.0001$) were associated with a worse OS. When we analyzed the treatment modalities in combinations, any treatment combination that included surgery was associated with a better OS compared with treatment combinations that included radiotherapy or chemotherapy without surgery.

Among the 740 HNC patients, 239 (32.3%) were never/occasional drinkers, 402 (54.3%) were current drinkers, and 99 (13.4%) were former drinkers. The Kaplan–Meier plots indicated that prediagnosis alcohol use, including higher frequency, amount, and drink-years, was associated with a worse OS of HNC patients (Supplementary Figs. S1–S4). Ever (former + current) alcohol drinking before HNC diagnosis was associated with a worse OS of HNC patients (HR = 1.45; 95% CI, 1.04–2.02; $P = 0.03$) in the unadjusted Cox proportional hazards model (Model 1; Table 2). The unadjusted model showed that higher frequency, amount, and drink-years of alcohol drinking were associated with a worse OS of HNC patients with a significant trend ($P < 0.05$). After adjusting for stage (Model 2), the association between prediagnosis alcohol use and the OS of HNC patients was substantially attenuated, whereas further adjustment with grade (Model 3) did not change the HRs by more than 10%. Additional adjustment with treatment (Model 4) further moved the HRs toward the null.

Prediagnosis alcohol drinking was associated with a higher stage of HNC (Supplementary Table S1). Sixty-one percent of HNC patients who ever drank alcohol were diagnosed with stage 3 or 4 HNC, whereas 49% of non–alcohol-drinking HNC patients were diagnosed with stage 3 or 4 HNC ($P = 0.003$). Alcohol drinking was associated with both a higher T stage and N stage, indicating that alcohol drinking may influence both the growth and dissemination of HNC. HNC grade did not differ significantly by alcohol drinking status ($P = 0.89$; Supplementary Table S1).

The stage of HNC was associated with treatment modality (Supplementary Table S2). More early-stage (stages 1 and 2) patients underwent surgery than late-stage (stages 3 and 4) patients (84% vs. 59%, $P < 0.0001$), whereas more late-stage patients received radiotherapy (77% vs. 16%, $P < 0.0001$) and chemotherapy (71% vs. 6%, $P < 0.0001$) compared with early-stage patients.

Based on the above results, we drew a directed acyclic graph to illustrate the relationship between prediagnosis alcohol use and the clinical characteristics of the HNC patients and their roles in the OS of HNC patients (Fig. 1). Our data suggested that the association between prediagnosis alcohol use and the poorer OS of HNC patients was mainly through the influence of alcohol on the stage of HNC, with alcohol-drinking HNC patients more likely to present with late-stage HNC at diagnosis. The stage of HNC may then affect the OS of HNC patients by determining the different

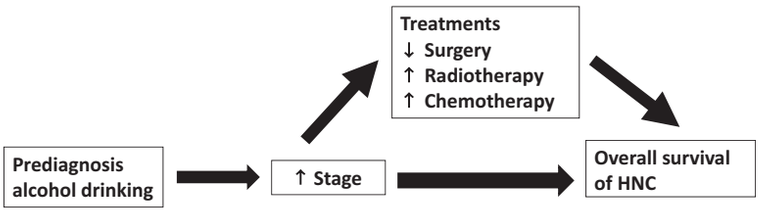

Figure 1.
A directed acyclic graph illustrating the relationship between prediagnosis alcohol use and the clinical characteristics of the HNC patients and their roles in the OS of the HNC patients.

treatment modalities and by other mechanisms. This also suggested that stage and treatment are intermediate variables on the causal pathway between prediagnosis alcohol use and the OS of HNC patients. Therefore, in our subsequent analyses we did not include stage and treatment as covariates in the Cox proportional hazards models for assessing the influence of prediagnosis alcohol use on the OS of HNC patients.

The association between prediagnosis alcohol drinking and the OS of HNC patients differed by the HNC subsite (Table 3). Prediagnosis alcohol use was not significantly associated with the OS of oral cancer but was significantly associated with a poorer OS of the other subsites of HNC combined (cancers of the oropharynx, hypopharynx, and larynx). In addition, prediagnosis alcohol use was associated with a higher cancer stage only for cancers of the oropharynx, hypopharynx, and larynx but not for oral cancer (Supplementary Table S1). This further gave support that the poor survival associated with prediagnosis alcohol use was mainly through the association between prediagnosis alcohol use and higher cancer stage.

Among the 370 HNC patients carrying the fast *ADH1B*2/*2* genotype, 233 (63%) were ever alcohol drinkers [45 (12.2%) former drinkers and 188 (50.8%) current drinkers], whereas among the 347 individuals carrying the slow *ADH1B* genotypes **1/*2* or **1/*1*, 254 (73.2%) were ever alcohol drinkers [48 (13.8%) former drinkers and 206 (59.4%) current drinkers]. Prediagnosis alcohol drinking was associated with a significantly worse OS among HNC patients with the *ADH1B*2/*2* genotype, which is associated with a faster ADH1B enzyme activity, whereas no significant association between prediagnosis alcohol drinking and the OS of HNC was observed among HNC patients with the slow *ADH1B* genotypes **1/*2* and **1/*1* (Table 4). Among the 302 HNC patients with the normal-function *ALDH2*1/*1* genotype, 233 (77.2%) were ever alcohol drinkers [45 (14.9%) former drinkers and 188 (62.3%) current drinkers], whereas among the 415 HNC patients carrying the slow (**1/*2*)/nonfunctional (**2/*2*) *ALDH2* genotypes, 254 (61.2%) were ever alcohol drinkers [48 (11.6%) former drinkers and 206 (49.6%) current drinkers]. HNC patients with the slow (**1/*2*) or nonfunctional (**2/*2*) *ALDH2* genotypes had a significantly worse OS associated with prediagnosis alcohol drinking, whereas no significant association between prediagnosis alcohol drinking and the OS of HNC was observed among HNC patients with the *ALDH2*1/*1* genotype, which confers a normal ALDH2 enzyme activity (Table 4). Although HNC patients with the fast *ADH1B*2/*2* genotype or the slow (**1/*2*)/nonfunctional (**2/*2*) *ALDH2* genotypes drank significantly ($P < 0.05$) less in frequency and amount (Table 4), our results indicated that they experienced a higher mortality risk associated with alcohol drinking. The same frequency or amount of alcohol consumption was associated with a higher risk of mortality among HNC patients carrying the fast *ADH1B*2/*2* genotype or the slow (**1/*2*)/nonfunctional (**2/*2*) *ALDH2* genotypes. When we combined *ADH1B* and *ALDH2* for analysis (Table 5; Supplementary Fig. S5), HNC patients who drank alcohol and had the fast *ADH1B* and the slow/nonfunctional *ALDH2* genotype combination showed a worse OS compared with all other HNC patients (HR = 1.88; 95% CI, 1.33–2.64; $P = 0.0003$). Furthermore, HNC patients who drank alcohol and had the fast *ADH1B* and the slow/nonfunctional *ALDH2* genotype combination had the highest percentage of late overall stage (73% with stage 3 or 4), T stage (52% with T stage 3 or 4), and N stage (56% with N stages 1 to 3) compared with all other HNC patients (the percentages of overall stages 3 and 4 ranged from 43%–62%, the percentages of T stages 3 and 4 ranged from 26%–43%, and the percentages of N stages 1 to 3 ranged from 31%–50%; Supplementary Table S3).

Discussion

Our analysis showed a significant positive dose–response relationship between prediagnosis alcohol use and a worse OS of HNC. This association was more significant for non–oral cavity HNC than for oral cancer. The association between prediagnosis alcohol use and the poorer OS of HNC patients was mainly through its association with a higher stage of HNC at diagnosis. The worst OS associated with alcohol use was observed among HNC patients with the fast *ADH1B* and the slow/nonfunctional *ALDH2* genotype combination.

Among the ten published studies on the relationship between prediagnosis alcohol use and HNC survival (7–15), three showed no significant association (11, 14, 15). The other seven studies along with the results of our analysis all observed a worse survival of HNC associated with prediagnosis alcohol use (7–10, 12, 13, 16); however, there was inconsistency with regards to the amount of alcohol use that may result in a poorer survival of HNC patients. Three previous studies and our study found that heavy drinking was associated with a worse survival of HNC patients (7, 9, 16), whereas three other studies found a worse survival of HNC patients with light to moderate drinking (10, 12, 13). Overall, most studies found that the survival was poorer among HNC patients who reported prediagnosis alcohol drinking. More investigations are needed to clarify the relationship between the level of alcohol use and the survival of HNC patients.

Our results indicated that prediagnosis alcohol use was associated with a higher stage of HNC at diagnosis. There are two possible explanations: (1) alcohol may promote the growth and progression of HNC; and (2) alcohol use may be associated with a delay in seeking HNC treatment. Most mechanistic studies have focused on the influence of alcohol on cancer formation. Only a few studies have examined the role of alcohol in the growth and the metastasis of cancer, and most of these studies focused on cancers other than HNC (19). Although some of these studies indicated that alcohol use may interact with the immune response to influence tumor metastasis and survival, the results so far have been inconclusive (19). The mechanistic studies on the

Table 3. The association between prediagnosis alcohol use and the OS of HNC patients by subsite

Prediagnosis alcohol use	Oral cancer			Oropharyngeal + hypopharyngeal cancer			Laryngeal cancer			Oropharyngeal + hypopharyngeal cancer + laryngeal cancer		
	n (%)	HR (95% CI)[a]	*P*	*n* (%)	HR (95% CI)[a]	*P*	*n* (%)	HR (95% CI)[a]	*P*	*n* (%)	HR (95% CI)[a]	*P*
Never + occasional	177 (37.90)	Referent		29 (16.48)	Referent		32 (37.21)	Referent		61 (23.28)	Referent	
Former	56 (11.99)	1.29 (0.69–2.40)	0.42	30 (17.05)	2.28 (0.93–5.60)	0.07	9 (10.47)	1.54 (0.30–7.96)	0.61	39 (14.89)	2.56 (1.22–5.37)	0.01
Current	234 (50.11)	1.10 (0.72–1.69)	0.66	117 (66.48)	1.55 (0.70–3.44)	0.28	45 (52.33)	1.39 (0.47–4.15)	0.55	162 (61.83)	1.81 (0.97–3.38)	0.06
Former + current	290 (62.10)	1.14 (0.75–1.71)	0.54	147 (83.53)	1.69 (0.77–3.69)	0.19	54 (62.80)	1.42 (0.49–4.07)	0.52	201 (76.72)	1.95 (1.06–3.59)	0.03
Frequency												
Never	160 (34.26)	Referent		26 (14.77)	Referent		27 (31.40)	Referent		53 (20.23)	Referent	
Monthly	17 (3.64)	0.25 (0.03–1.83)	0.17	3 (1.70)	—	—	5 (5.81)	1.34 (0.15–12.03)	0.79	8 (3.05)	0.51 (0.07–3.94)	0.52
Weekly	43 (9.21)	1.18 (0.60–2.32)	0.64	23 (13.07)	0.87 (0.29–2.58)	0.79	9 (10.47)	1.66 (0.30–9.06)	0.56	32 (12.21)	1.24 (0.50–3.09)	0.64
Daily	232 (49.68)	1.08 (0.70–1.66)	0.73	122 (69.32)	1.51 (0.68–3.32)	0.31	44 (51.16)	1.49 (0.46–4.83)	0.51	166 (63.36)	1.90 (1.00–3.61)	0.05
Unknown	15 (3.21)	—	—	2 (1.14)	—	—	1 (1.16)	—		3 (1.15)	—	
		P trend = 0.58			*P* trend = 0.13			*P* trend = 0.51			*P* trend = 0.02	
Amount[b]												
Never	147 (35.34)	Referent		24 (14.91)	Referent		26 (32.91)	Referent		50 (20.83)	Referent	
Light	63 (15.14)	0.87 (0.45–1.70)	0.69	24 (14.91)	0.78 (0.25–2.42)	0.66	10 (12.66)	1.98 (0.33–11.88)	0.46	34 (14.17)	1.26 (0.48–3.26)	0.64
Moderate	58 (13.94)	1.29 (0.69–2.38)	0.43	29 (18.01)	1.13 (0.41–3.12)	0.81	7 (8.86)	2.58 (0.43–15.48)	0.30	36 (15.00)	1.81 (0.76–4.29)	0.18
Heavy	133 (31.97)	1.02 (0.62–1.69)	0.92	82 (50.93)	1.71 (0.72–4.08)	0.22	35 (44.30)	1.73 (0.43–6.94)	0.44	117 (48.75)	2.23 (1.09–4.59)	0.03
Unknown	15 (3.61)	—	—	2 (1.24)	—	—	1 (1.27)	—	—	3 (1.25)	—	—
		P trend = 0.76			*P* trend = 0.05			*P* trend = 0.46			*P* trend = 0.01	
Drink-years[c]												
0	147 (35.34)	Referent		24 (14.91)	Referent		26 (32.91)	Referent		50 (20.83)	Referent	
0.1–54.9	94 (22.60)	0.88 (0.49–1.58)	0.67	41 (25.47)	1.09 (0.42–2.85)	0.85	10 (12.66)	2.88 (0.58–14.28)	0.20	51 (21.25)	1.79 (0.80–4.02)	0.16
55–159.9	75 (18.03)	1.24 (0.70–2.19)	0.47	46 (28.57)	1.17 (0.45–3.02)	0.75	18 (22.78)	1.06 (0.18–6.33)	0.95	64 (26.67)	1.52 (0.68–3.41)	0.31
160 or more	73 (17.55)	1.10 (0.62–1.97)	0.74	46 (28.57)	1.94 (0.78–4.79)	0.15	22 (27.85)	2.32 (0.55–9.72)	0.25	68 (28.33)	2.55 (1.20–5.43)	0.01
Unknown	27 (6.49)	—	—	4 (2.48)	—	—	3 (3.80)			7 (2.92)	—	
		P trend = 0.54			*P* trend = 0.07			*P* trend = 0.39			*P* trend = 0.02	

Abbreviation: *N*, number.

[a]HR and 95% CI were calculated using Cox proportional hazards model.

[b]Light: < 14 g/day; moderate: 14–42 g/day; heavy: > 42 g/day.

[c]1 drink-year = 1 drink (14 grams of alcohol) per day x 1 year.

Table 4. The association between prediagnosis alcohol use and the OS of HNC patients by *ADH1B* genotypes or *ALDH2* genotypes

	ADH1B						*ALDH2*					
	TT (*2/*2) (fast)			CT (*1/*2) + CC (*1/*1) (slow)			GG (*1/*1) (normal)			AG (*1/*2) (slow) + AA (*2/*2) (nonfunctional)		
Prediagnosis alcohol use	***n* (%)**	**HR (95% CI)[a]**	***P***	***n* (%)**	**HR (95% CI)[a]**	***P***	***n* (%)**	**HR (95% CI)[a]**	***P***	***n* (%)**	**HR (95% CI)[a]**	***P***
Never + occasional	137 (37.03)	Referent		93 (26.80)	Referent		69 (22.85)	Referent		161 (38.80)	Referent	
Former	45 (12.16)	1.88 (1.00–3.56)	0.05	48 (13.83)	1.38 (0.70–2.71)	0.35	45 (14.90)	1.05 (0.49–2.25)	0.91	48 (11.57)	2.23 (1.24–3.98)	0.007
Current	188 (50.81)	1.78 (1.13–2.82)	0.01	206 (59.37)	0.96 (0.57–1.60)	0.86	188 (62.25)	1.04 (0.59–1.83)	0.91	206 (49.64)	1.56 (1.01–2.41)	0.04
Former + current	233 (62.97)	1.80 (1.15–2.81)	0.01	254 (73.20)	1.03 (0.63–1.70)	0.90	233 (77.15)	1.04 (0.60–1.80)	0.90	254 (61.21)	1.68 (1.10–2.54)	0.02
Frequency												
Never	124 (33.51)	Referent		81 (23.34)	Referent		56 (18.54)	Referent		149 (35.90)	Referent	
Monthly	13 (3.51)	0.32 (0.04–2.33)	0.26	12 (3.46)	0.32 (0.04–2.39)	0.27	13 (4.30)	0.57 (0.13–2.51)	0.46	12 (2.89)	—	—
Weekly	40 (10.81)	1.55 (0.78–3.08)	0.21	34 (9.80)	0.80 (0.34–1.89)	0.61	38 (12.58)	0.89 (0.38–2.05)	0.78	36 (8.67)	1.41 (0.69–2.88)	0.34
Daily	185 (50.00)	1.74 (1.10–2.77)	0.02	210 (60.52)	0.99 (0.59–1.66)	0.96	183 (60.60)	1.01 (0.56–1.83)	0.97	212 (51.08)	1.56 (1.02–2.40)	0.04
Unknown	8 (2.16)	—	—	10 (2.88)	—	—	12 (3.97)	—	—	6 (1.45)	—	—
		P trend = 0.01			*P* trend = 0.86			*P* trend = 0.81			*P* trend = 0.02	
Amount[b]												
Never	122 (35.78)	Referent		67 (22.11)	Referent		53 (19.63)	Referent		136 (36.36)	Referent	
Light	51 (14.96)	1.39 (0.72–2.69)	0.33	43 (14.19)	0.45 (0.17–1.23)	0.12	44 (16.30)	0.70 (0.29–1.68)	0.42	50 (13.37)	1.08 (0.54–2.18)	0.83
Moderate	42 (12.32)	1.81 (0.95–3.45)	0.07	53 (17.49)	0.98 (0.46–2.08)	0.96	48 (17.78)	1.08 (0.50–2.33)	0.85	47 (12.57)	1.56 (0.82–2.97)	0.18
Heavy	117 (34.31)	1.59 (0.94–2.67)	0.08	131 (43.23)	1.15 (0.64–2.07)	0.64	112 (41.48)	1.01 (0.53–1.94)	0.98	136 (36.36)	1.65 (1.02–2.67)	0.04
Unknown	9 (2.64)	—	—	9 (2.97)	—	—	13 (4.81)	—	—	5 (1.34)	—	—
		P trend = 0.07			*P* trend = 0.30			*P* trend = 0.71			*P* trend = 0.03	
Drink-years[c]												
0	122 (35.78)	Referent		67 (22.11)	Referent		53 (19.63)	Referent		136 (36.36)	Referent	
0.1–54.9	76 (22.29)	1.45 (0.81–2.59)	0.21	65 (21.45)	0.74 (0.35–1.56)	0.42	58 (21.48)	0.78 (0.36–1.71)	0.53	83 (22.19)	1.34 (0.76–2.35)	0.31
55–159.9	71 (20.82)	1.87 (1.06–3.29)	0.03	70 (23.10)	0.76 (0.37–1.58)	0.47	67 (24.81)	1.22 (0.61–2.46)	0.57	74 (19.79)	1.19 (0.65–2.19)	0.57
160 or more	58 (17.01)	1.50 (0.80–2.78)	0.20	81 (26.73)	1.38 (0.74–2.58)	0.31	69 (25.56)	0.95 (0.46–1.95)	0.88	70 (18.72)	2.04 (1.19–3.49)	0.01
Unknown	14 (4.11)	—	—	20 (6.60)	—	—	23 (8.52)	—	—	11 (2.94)	—	—
		P trend = 0.09			*P* trend = 0.23			*P* trend = 0.81			*P* trend = 0.02	

[a]HR and 95% CI were calculated using Cox proportional hazards model.
[b]Light: < 14 g/day; moderate: 14–42 g/day; heavy: > 42 g/day.
[c]1 drink-year = 1 drink (14 grams of alcohol) per day × 1 year.

Table 5. The OS of HNC patients by prediagnosis alcohol drinking status and combinations of *ADH1B* and *ALDH2* genotypes

Prediagnosis alcohol use	ADH1B and ALDH2 genotype combination	*n* (%)	HR (95% CI)[a]	*P*
Never + occasional	Group 1: Fast *ADH1B* (*2/*2) + Fast *ALDH2* (*1/*1)	43 (6.00)	Referent	
Never + occasional	Group 2: Fast *ADH1B* (*2/*2) + Slow *ALDH2* (*1/*2+*2/*2)	94 (13.11)	0.69 (0.31–1.53)	0.36
Never + occasional	Group 3: Slow *ADH1B* (*1/*1 +*1/*2) + Fast *ALDH2* (*1/*1)	26 (3.63)	0.96 (0.35–2.64)	0.93
Never + occasional	Group 4: Slow *ADH1B* (*1/*1 +*1/*2) + Slow *ALDH2* (*1/*2+*2/*2)	67 (9.34)	0.90 (0.40–2.01)	0.80
Ever	Group 1: Fast *ADH1B* (*2/*2) + Fast *ALDH2* (*1/*1)	122 (17.02)	1.13 (0.56–2.29)	0.73
Ever	Group 2: Fast *ADH1B* (*2/*2) + Slow *ALDH2* (*1/*2 +*2/*2)	111 (15.48)	1.77 (0.89–3.52)	0.11
Ever	Group 3: Slow *ADH1B* (*1/*1 +*1/*2)+ Fast *ALDH2* (*1/*1)	111 (15.48)	0.90 (0.43–1.88)	0.78
Ever	Group 4: Slow *ADH1B* (*1/*1 +*1/*2)+ Slow *ALDH2* (*1/*2+*2/*2)	143 (19.94)	0.98 (0.49–1.99)	0.96
Ever alcohol drinkers + Group 2: Fast *ADH1B* (*2/*2) + Slow *ALDH2* (*1/*2 +*2/*2) vs. all others			1.88 (1.33–2.64)	0.0003

[a]HR and 95% CI were calculated using Cox proportional hazards model.

relationship between alcohol use and HNC growth and progression are even more limited. Saad and colleagues identified 8 miRNAs that were significantly upregulated in alcohol-related HNC (20). *In vitro* experiment with the HNC cell lines showed that miR-30a and miR-934 were the most significantly upregulated after being exposed to alcohol and acetaldehyde (20). These two miRNAs were associated with HNC cell proliferation and invasion (20). The association between alcohol use and the higher stage HNC may also be explained by treatment delay. Alcohol use has been associated with a delay in seeking HNC treatment (21), which may result in the higher stage of HNC at diagnosis. Overall, information is limited on the biological and behavioral impact of prediagnosis alcohol use on the survival of HNC patients, and more investigations are required.

Our results showed that prediagnosis alcohol drinking was associated with the worst survival among HNC patients with the fast *ADH1B*2/*2* and the slow (**1/*2*) or nonfunctional (**2/*2*) *ALDH2* genotype combination. Although the biological mechanisms for this observation are yet to be elucidated, the rapid generation and the slow metabolism of acetaldehyde after alcohol consumption for these individuals may possibly contribute to the growth and progression of HNC. This is supported by our results showing that HNC patients with the fast *ADH1B* and the slow/nonfunctional *ALDH2* genotype combination had the highest percentage of late-stage diagnosis associated with prediagnosis alcohol use compared with HNC patients with other *ADH1B* and *ALDH2* genotype combinations. This suggested that the association between prediagnosis alcohol drinking and the higher stage of HNC at diagnosis may have a biological explanation rather than being caused by the delay in seeking medical treatment because the genotypes of *ADH1B* and *ALDH2* are unlikely to affect one's health-seeking behaviors. Only two other studies have examined the influence of ethanol-metabolizing genes on the association between alcohol consumption and the survival of HNC patients. Avincsal and colleagues found that HNC patients who were heavy drinkers and carried *ALDH2*2* allele had a worse survival of HNC compared with other patients, whereas *ADH1B* polymorphism did not modify the relationship between alcohol use and the survival of HNC patients (14). In contrast, Kawakita and colleagues reported a significant association between prediagnosis alcohol use and a worse disease-free survival of HNC only among patients carrying the *ALDH2*1/*1* genotype (11). Given the inconsistencies across the limited number of studies, more investigations are needed to determine the interaction between alcohol drinking and the polymorphisms of ethanol-metabolizing genes on the survival of HNC patients.

Our analysis showed that prediagnosis alcohol use was more strongly associated with a worse survival of non–oral cavity HNC than that of oral cancer. Dikshit and colleagues studied lifestyle habits and the prognosis of laryngeal and hypopharyngeal cancers, and found that among all these subsites of HNC, cancer of the epilarynx showed the strongest association between a poorer survival and prediagnosis alcohol use (7). Leoncini and colleagues found a significant association between prediagnosis alcohol use and a worse survival of all HNC subsites combined, but the association was not statistically significant for the subsite analysis, likely due to the smaller sample size for each subsite (10). Giraldi and colleagues found that prediagnosis alcohol use was associated with a worse OS and HNC-specific survival of laryngeal cancer (12). Given the limited number of studies on the relationship between prediagnosis alcohol use and the survival of HNC by subsites, more investigations are needed.

This study has several limitations. The HNC patients were asked about their past alcohol consumption before the diagnosis of HNC, and the recall errors might have affected the precision and the accuracy of the effect estimates. Similarly, the smaller sample size in the subsite analysis may also have increased the imprecision of the effect estimates and the probability of a chance finding. Because of the case–control design, we could not be absolutely certain that the prediagnosis alcohol use was the antecedent event for the higher stage of HNC. However, we found that HNC patients with the fast *ADH1B* and the slow/nonfunctional *ALDH2* genotype combination had the highest percentage of late-stage diagnosis associated with prediagnosis alcohol use compared with HNC patients with other *ADH1B* and *ALDH2* genotype combinations. Because individuals were born with these genetic polymorphisms, the joint effect of fast *ADH1B* and the slow/nonfunctional *ALDH2* genotype combination and prediagnosis alcohol use likely preceded the growth and the progression of HNC. Finally, we did not adjust for HPV status in our analysis due to the lack of access to the tumor tissues to test for HPV. Although HPV-positive HNC has a more favorable prognosis (22), a study by Lupato and colleagues reported no association between alcohol use and oral HPV infection (23), thus HPV status may not be a confounder in the relationship between alcohol use and the survival of HNC patients. In contrast, alcohol use was associated with an increased risk of genital HPV infection according to Schabath and colleagues (24). Assuming this also applies to oral HPV infection and given the more favorable prognosis of HPV-related HNC, not adjusting for HPV status in our analysis might have underestimated the association between alcohol use and a worse survival of HNC patients.

The major strength of the study was that we showed a significant gene–environment interaction between prediagnosis alcohol use and the polymorphisms of *ADH1B* and *ALDH2* on the survival of HNC patients. This gene–environment interaction indicated that the association between prediagnosis alcohol use and a worse survival of HNC patients may have a biological explanation.

In conclusion, our study showed that prediagnosis alcohol consumption was associated with a worse survival of HNC patients. This was likely due to the association between prediagnosis alcohol use and a higher stage of HNC at diagnosis. Furthermore, individuals with the fast *ADH1B* and the slow/nonfunctional *ALDH2* genotype combination had a worse survival of HNC associated with prediagnosis alcohol drinking compared with those with other genotype combinations. Due to the limited number of studies, more investigations are needed to confirm these findings. Furthermore, the biological mechanisms underlying the association between prediagnosis alcohol use and the survival of HNC patients need to be explored.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Disclaimer

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Authors' Contributions

Conception and design: J.S. Chang
Development of methodology: J.S. Chang
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): W.-T. Lee, J.-R. Hsiao, C.-Y. Ou, C.-C. Huang, C.-C. Chang, S.-T. Tsai, K.-C. Chen, J.-S. Huang, T.-Y. Wong, Y.-H. Wu, W.-T. Hsueh, S.-Y. Wu, C.-J. Yen, C.-L. Lin, Y.-L. Weng, H.-C. Yang, Y.-S. Chen, J.S. Chang
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J.S. Chang
Writing, review, and/or revision of the manuscript: W.-T. Lee, J.-R. Hsiao, K.-C. Chen, J.-S. Huang, W.-T. Hsueh, S.-Y. Wu, C.-J. Yen, C.-L. Lin, Y.-L. Weng, H.-C. Yang, Y.-S. Chen, J.S. Chang
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): T.-Y. Wong, Y.-H. Lai, J.-Y. Chang, J.S. Chang
Study supervision: J.S. Chang

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