Research Article

Cancer Prevention Research

Alcohol-metabolizing Enzymes' Gene Polymorphisms and Susceptibility to Multiple Head and Neck Cancers

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Abstract

Multiple primary tumors (MPT), especially in the hypopharynx and esophagus, are challenging in patients with head and neck cancer (HNC). Alcohol and alcohol-metabolizing genes were reported to be related to upper digestive tract cancers. Here, we investigated whether the genotypes of alcohol-metabolizing enzymes (ADH1B, ADH1C, and ALDH2) affected patients' susceptibility to developing MPTs. We recruited 659 male patients with HNC between March 1996 and February 2017. Age- and gender-matched controls were also recruited. A total of 164 patients with HNC were identified to have second or third malignancies. The single-nucleotide polymorphisms in *ADH1B* (rs1229984), *ADH1C* (rs698), and *ALDH2* (rs671) were analyzed by TaqMan assays. The prevalence of *ALDH2* **2* allele carriers is significantly higher than that of **1*1* homozygotes for oral cavity ($P = 0.013$) and oropharyngeal cancers ($P = 0.012$). For *ADH1B*, the number of **1* allele carriers is significantly higher than that of **2*2* homozygotes for oropharyngeal ($P = 0.017$) and hypopharyngeal cancers ($P < 0.001$). *ADH1C* (rs698) SNPs are not significantly associated with tumor subsites (all $P > 0.05$). Polymorphisms in *ALDH2* (**2* allele carriers) and *ADH1B* (**1* allele carriers) significantly increase the risk of developing MPTs in the upper digestive tract [$P < 0.001$, OR (95% confidence interval (CI): 5.186 (2.444–11.004) and $P < 0.05$, OR (95% CI): 2.093 (1.149–3.812), respectively]. *ALDH2* (rs671) **2* and *ADH1B* (rs1229984) **1* allele carriers were shown to develop MPTs in the upper digestive tract. Genetic information may be used to identify high-risk patients for the development of MPTs.

Introduction

Head and neck squamous cell carcinoma is the sixth leading cancer by incidence worldwide. These cancers are related with environmental exposures of tobacco, betel quid (BQ), alcohol consumption and human papillomavirus (HPV; ref. 1). Oral cavity cancer (OCC) is the fourth most frequent malignancy in men in Taiwan (2), and its incidence has increased in recent years. The main reasons are the increased consumption of cigarettes, alcohol, and BQ. Because of the abovementioned environmental exposures, patients with head and neck cancer (HNC) have higher risks of multiple primary tumors (MPT) in Taiwan (3). Some of the secondary primary tumors occur in the oral cavity, while others occur in the oropharynx, hypopharynx, and esophagus. In patients with HNC, ablative surgery and specific postoperative oncologic treatment (irradiation and chemotherapy) of head and neck cancer (HNC), especially oral cancer, could alter oral cavity anatomic structures and functions. The main consequence of radiotherapy is radiation-induced skin fibrosis of the neck. Clinically, the early detection of lesions in the oropharynx or esophagus is challenging. MPTs at these sites are usually diagnosed at late stages, and their prognoses are poor. To detect lesions earlier, routine screening including CT scans and panendoscopy exams is essential. However, the frequency of regular exams, the detection rate, and the resultant costs are the main concerns in the clinic. Recently, "precision preventive medicine" incorporated genetic

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information, such as BRCA1/BRCA2, into breast cancer prevention program (4). The risks of breast cancer were significantly reduced in high-risk women and *BRCA* mutation carriers who received prophylactic mastectomy and salpingo-oophorectomy (5, 6). For those with higher risks of MPTs, more frequent and sensitive examinations should be arranged. This screening would be cost-effective and increase the early detection rate in patients with HNC.

Alcohol was demonstrated to be related to esophageal cancer (7). In the upper digestive tract, alcohol consumption and its effects on carcinogenesis are important. One such effect is from alcohol metabolites, and the others are due to ethanol serving as a solvent for carcinogens, which cause upper aerodigestive tract cancers (8, 9). To investigate the susceptibility for upper digestive tract cancers, we focused our study on alcohol-metabolizing genes.

There are two steps in metabolizing ethanol: one is the conversion of ethanol to acetaldehyde, and the second is the conversion from acetaldehyde to acetate. Alcohol dehydrogenase (ADH) enzymes are responsible for the first step, whereas aldehyde dehydrogenases (ALDH) are responsible for the second step. In the literature, the *ADH1B* Arg48His (rs1229984) and Arg370Cys (rs2066702) polymorphisms, the *ADH1C* Arg272Gln (rs1693482) and Ile350Val (rs698) polymorphisms, and the *ALDH2* Glu504Lys (rs671) polymorphism were reported to modify the activity of these enzymes and to be associated with the increased risk of HNC and esophageal cancer, especially in Asians (10–14). Although the association between these functional genetic polymorphisms and HNC risk has been investigated, to our knowledge, there has been no study concerning the roles of these polymorphisms in occurrence of MPTs (12, 15–17).

To clarify the association between genetic polymorphisms in alcohol-metabolizing genes and the occurrence of MPTs, we retrospectively collected a group of patients with HNC with tumors at different subsites. In addition, we identified a subset of patients who developed multiple primary cancers at the time of diagnosis or during follow-up. We investigated the distributions of *ADH1B*, *ADH1C*, and *ALDH2* SNP genotypes among tumor subsites. High-risk genotypes for MPTs were also analyzed in this study.

Materials and Methods

Patients

The study was approved by the institutional review board of Chang Gung Medical Foundation (Taoyuan, Taiwan). The approval date was January 10, 2017 and the approval number was 201601884B0. This study was conducted in accordance with Declaration of Helsinki. We recruited 659 male patients with HNC treated between 1996 and 2016 at Chang Gung Memorial Hospital (Linkou, Taiwan). All the histology of the primary HNCs were squamous cell carcinoma. The definition of oral cavity cancer includes cancer of the tongue, bucca, alveolus, retromolar space, hard palate, lip, and mouth floor. Oropharyngeal cancer includes cancer of the soft palate and oropharyngeal walls. The tonsil is separated as a subsite. Hypopharyngeal cancer includes cancer of the lateral pharyngeal wall, pyriform sinus, and posterior cricoid region. All the patients were followed regularly in the clinic for more than 2 years. Patients with second primary tumors were confirmed by biopsy, and the possibility of recurrence or metastasis was excluded. Multiple primary tumors (MPT) were categorized into 4 types: 1, all MPTs occurring in the oral cavity; 2, HNC with MPTs occurring in different upper digestive locations, such as the oral cavity, oropharynx, and esophagus; 3, HNC with MPTs in upper respiratory tract locations, such as the larynx or lung; and 4, HNC with MPTs not related to the aerodigestive tract, such as prostate cancer, hepatoma, or lymphoma. Furthermore, 427 healthy controls without a history of cancer were enrolled from a random sample of the Taiwanese general population (18). All patients signed informed consent forms. The participants were interviewed with the questionnaire, which included questions on general demographic data, family history, and the habitual use of cigarettes, alcohol, and BQ.

Specimen collection and DNA extraction

Every participant was requested to draw 10 mL venous blood in a Vacutainer, which contained an anticoagulant. The buffy coat was then isolated and preserved in a −80°C freezer. High molecular weight DNA was isolated from the buffy coat cells using a conventional phenol–chloroform method (18).

Genotyping of *ADH1B*, *ADH1C*, and *ALDH2*

Genotyping for *ADH1B* rs1229984 (assay ID: C_2688467_20), *ADH1B* rs2066702 (assay ID: C_ 11941896_20), *ADH1C* rs1693482 (assay ID: C_2688502_10), *ADH1C* rs698 (assay ID: C_26457410_10), and *ALDH2* rs671 (assay ID: C_11703892_10) was based on TaqMan SNP genotyping assays (Thermo Fisher Scientific, Inc). Reactions were performed in 10-μL volumes containing 10 ng DNA, 2 × TaqMan Genotyping Master Mix (Thermo Fisher Scientific, Inc), and 20 × TaqMan Drug Metabolism SNP Genotyping Assay Reagent Mix (Thermo Fisher Scientific, Inc). The no-template control (NTC) was included in each 96-well reaction plate. The manufacturer's standard recommendations were followed with regard to cycling conditions. The device used for analysis was a 7500 Fast Real-Time PCR System with built-in v2.0.6 software for SNP genotyping (Thermo Fisher Scientific, Inc.). Ten percent of the study subjects were randomly selected for the genotyping of each polymorphism to examine the reliability of the genotyping assays. Direct sequencing was also performed to confirm the genotyping results. *ADH1B* haplotypes for the Arg48His (rs1229984) and Arg370Cys (rs2066702) polymorphisms were defined as **1* for the

wild-type allele (Arg48 and Arg370) and *2 and *3 for the His48 and Cys370 variants, respectively. The *ADH1C* haplotypes were defined as Arg272Gln (rs1693482) and Ile350Val (rs698). Whereas these two polymorphisms showed perfect linkage disequilibrium (LD) among subjects with Pacific Rim heritage (19), in this study, the haplotypes were defined as *1 for the Arg272 or Ile350 polymorphisms and *2 for the Gln272 or Val350 polymorphisms. The wild-type allele *ALDH2* *1 and variant allele *2 were defined as Glu504 and Lys504 (rs671). The distribution of genotype frequencies was tested for Hardy–Weinberg equilibrium to assess the quality of genotyping.

Statistical analysis

The Hardy–Weinberg equilibrium was tested for the SNPs in each gene with the χ^2 test. The distribution of *ADH1B*, *ADH1C*, and *ALDH2* genotypes was tested between the controls and HNC cases using the χ^2 test. The risks for *ADH1B*, *ADH1C*, and *ALDH2* genotypes to develop HNCs or MPTs were calculated by using logistic regression analyses for crude ORs and adjusted ORs when adjusting for age and alcohol drinking status. We investigated genotypes of 3 genes in this study. To deal with the probability of multiple tests, the significance *P* value in this study was set at 0.017 by the Bonferroni correction (0.05/n).

Results

Patients

The recruitment period was between March 1997 and February 2017. A total of 659 patients with HNC were included in this study. The mean follow-up period was 47.13 months (range: 0–291 months, SD: 46.28 months). There were 642 primary tumors located in the oral cavity (55.9%), oropharynx (14.3%), tonsil (9.9%), and hypopharynx (19.7%). Primary subsites of the other 17 patients were listed in Table 1.

We had 483 patients with primary HNC in whom we did not detect MPT lesions during follow-up (Table 1; Fig. 1). Eighty-nine patients had multiple oral primary tumors, and 57 had MPTs in the esophagus or upper gastrointestinal tract, of which 19 (33.3%) developed MPTs in the oropharynx or hypopharynx and 38 (66.7%) developed MPTs in the esophagus or gastrointestinal tract. In 11 patients, the MPTs developed in the lung or aerodigestive tract. Nineteen patients developed MPTs unrelated to the aerodigestive tract, which included prostate cancer, hepatoma, or nasopharyngeal carcinoma.

Table 1. Characteristics of the patients with HNC and control subjects

	Controls *N* = 427, *n* (%)	Cases *N* = 659, *n* (%)	*P*
Age	40.03 (±14.70)	51.61 (±10.64)	<0.001
Range	15.0–99.0	25.0–87.0	
Sex			
Male	238 (55.7)	659 (100.0)	<0.001
Female	189 (44.3)	0 (0)	
Alcohol consumption			
Yes	61 (14.3)	506 (76.8)	<0.001
No	364 (85.2)	153 (23.2)	
Unknown	2 (0.5)	0 (0)	
Cigarette smoking			
Yes	119 (27.9)	503 (76.3)	<0.001
No	306 (71.7)	156 (23.7)	
Unknown	2 (0.5)	0 (0)	
Betel quid chewing			
Yes	29 (6.8)	524 (79.5)	<0.001
No	397 (93.0)	135 (20.5)	
Unknown	1 (0.2)	0 (0)	
Tumor subsites			
Oral cavity		359 (55.7)	
Oropharynx		92 (14.0)	
Tonsil		64 (9.7)	
Hypopharynx		127 (19.3)	
Larynx		10 (1.5)	
Nasal cavity		3 (0.5)	
Skin		1 (0.2)	
Esophagus		2 (0.3)	
Unknown primary		1 (0.2)	
Distribution of MPTs			
Oral cancer without MPTs		483 (73.3)	
MPTs in oral cavity		89 (13.5)	
MPTs in esophageal or upper digestive tract		57 (8.6)	
MPTs in lung or aerodigestive tract		11 (1.7)	
Other MPTs		19 (2.9)	

Allele distribution of *ADH1B*, *ADH1C*, and *ALDH2* in normal controls

In this study, we recruited 427 normal controls for the analysis of *ADH1B*, *ADH1C*, and *ALDH2* (Table 2). The *ADH1B**1* allele was detected in 25.5% of the control group. The prevalences of the three genotypes were as follows: *ADH1B**1**1*: 26 (6.1%); **1**2*: 166 (38.9%); and **2**2*: 235 (55.0%). The *ADH1C**1* allele was detected in 90.2% of the controls. The prevalences of the *ADH1C* genotypes were as follows: *ADH1C**1**1*: 347 (81.3%); **1**2*: 76 (17.8%); and **2**2*: 5 (1.2%). The *ALDH2**1* allele was detected in 70.5% of the controls. The prevalence of the *ALDH2* genotypes were as follows: *ALDH2**1**1*: 215 (50.4%); *ALDH2**1**2*: 172 (40.3%); and *ALDH2**2**2*: 40 (9.4%). The distribution of these 3 alcohol-metabolizing genes did not deviate from the Hardy–Weinberg disequilibrium.

Allele distribution of *ADH1B*, *ADH1C*, and *ALDH2* in patients with HNC

As shown in Table 2, the prevalence of the *ADH1B* *1* allele is higher in patients with HNC than in normal controls (25.5%; $P < 0.001$). The prevalence of the *ADH1B* *1* allele according to tumor subsite was 24.5% in oral cavity cancer, 37.0% in oropharyngeal cancer, 40.6% in tonsillar cancer, and 43.3% in hypopharyngeal cancer. The prevalence of *1* allele carriers was significantly higher in

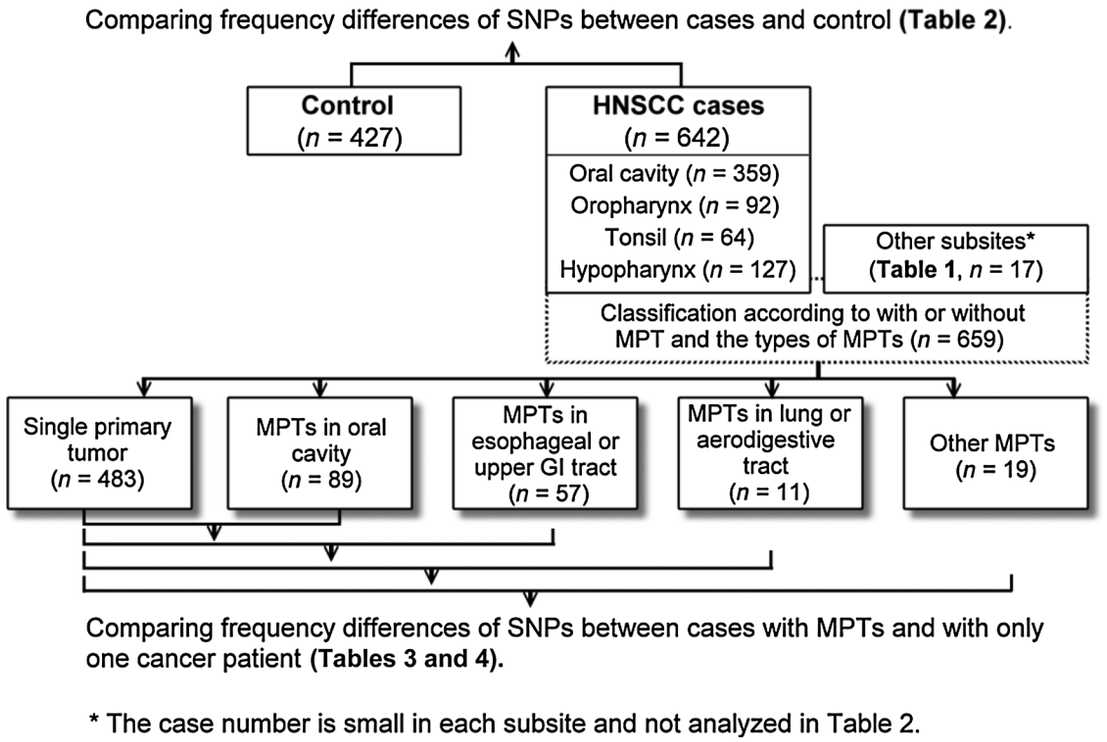

Figure 1.
Schema illustrating the patient distribution and data analysis in the study.

Table 2. Distribution of genotypes in alcohol-metabolizing enzyme genes in relation to tumor subsites

		Primary tumor subsites [*n* (%)]				
Genes	**Control [*n* (%)]**	**Oral cavity**	**Oropharynx**	**Tonsil**	**Hypopharynx**	***P***
ADH1B[a]						
Alleles						
*1 (slow)[b]	218 (25.5)	176 (24.5)	68 (37.0)	52 (40.6)	110 (43.3)	<0.001
*2 (fast)[b]	636 (74.5)	542 (75.5)	116 (63.0)	76 (59.4)	144 (56.7)	
Genotypes						
*1*1	26 (6.1)	26 (7.2)	14 (15.2)	15 (23.4)	29 (22.8)	<0.001
*1*2	166 (38.9)	124 (34.5)	40 (43.5)	22 (34.4)	52 (40.9)	
*2*2	235 (55.0)	209 (58.2)	38 (41.3)	27 (42.2)	46 (36.2)	
ADH1C						
Alleles						
*1 (fast)[b]	770 (90.2)	660 (91.9)	163 (88.6)	110 (85.9)	222 (87.4)	0.105
*2 (slow)[b]	84 (9.8)	58 (8.1)	21 (11.4)	18 (14.1)	32 (12.6)	
Genotypes						
*1*1	347 (81.3)	306 (85.2)	72 (78.3)	47 (73.4)	98 (77.2)	0.269
*1*2	76 (17.8)	48 (13.4)	19 (20.7)	16 (25.0)	26 (20.5)	
*2*2	4 (0.9)	5 (1.4)	1 (1.1)	1 (1.6)	3 (2.4)	
ALDH2						
Alleles						
*1 (active)[c]	602 (70.5)	496 (67.8)	123 (66.8)	88 (68.8)	178 (70.1)	0.745
*2 (inactive)[c]	252 (29.5)	236 (32.2)	61 (33.2)	40 (31.2)	76 (29.9)	
Genotypes						
*1*1	215 (50.4)	149 (41.5)	33 (35.9)	25 (39.1)	52 (40.9)	<0.001
*1*2	172 (40.3)	184 (51.3)	57 (62.0)	38 (59.4)	74 (58.3)	
*2*2	40 (9.4)	26 (7.2)	2 (2.2)	1 (1.6)	1 (0.8)	

[a]*ADH1B*: in all samples, codon 370 was in the CC form (Arg370), so only *ADH1B*1* and *ADH1B*2* were in the Taiwanese population; there were no *ADH1B*3* (Cys370) alleles in our study.
[b]Fast and slow indicate the metabolic speed of the allozyme that is encoded by the specific allele.
[c]Active and inactive indicate the enzyme activity of the allozyme that is encoded by the specific allele.

oropharyngeal (60.2% vs. 45.0%, $P = 0.016$) and hypopharyngeal cancers (63.7% vs. 45.0%, $P < 0.001$) than in normal controls. The allele proportion of *ADH1C* between HNC and normal controls was statistically nonsignificant ($P = 0.105$). Furthermore, the prevalence of the homozygous *ADH1C*1*1* allele was higher in patients with oral cavity cancer (85.2%) than in normal controls (81.3%); the prevalence of this allele in patients with cancers of the other three subsites, including the oropharynx (78.3%), tonsil (73.4%), and hypopharynx (77.2%), was even lower. The observations for *ADH1C* were statistically nonsignificant ($P = 0.269$). The prevalence of *ALDH2*2* allele carriers was significantly higher among patients with HNC than among normal controls (Table 2, $P < 0.001$). The prevalence of **2* allele carriers was the highest in patients with oropharyngeal (64.2%), tonsillar (61.0%), and hypopharyngeal cancers (59.1%), followed by patients with oral cavity cancer (58.5%).

Allele distribution of *ADH1B*, *ADH1C*, and *ALDH2* in patients with HNC with MPT occurrence

A total of 176 patients with HNC had an occurrence of MPTs, and 483 patients with HNC had a single primary tumor (Table 3). Overall, *ADH1B*1* allele carriers comprised 52.4% of patients with a single primary tumor, 30.3% of patients with multiple oral cavity cancers, 70.2% of patients with multiple aerodigestive tract cancers (including hypopharyngeal and esophageal cancers), 45.5% of patients with oral and lung cancers, and 26.4% of patients with oral and nonrelated multiple cancers ($P < 0.001$). Overall, the distribution of *ADH1C* genotypes among MPT subgroups had borderline statistical significance ($P = 0.042$). The prevalence of the *ADH1C*1*1* genotype was higher in patients with MPTs in the oral cavity (93.3%) than in those with a single primary tumor (80.5%). *ALDH2*2* allele carriers comprised 57.3% of patients with a single primary tumor, 61.8% of patients with multiple oral cavity cancers, 84.3% of patients with multiple aerodigestive tract cancers (including hypopharyngeal and esophageal cancers), 36.4% of patients with oral and lung cancers, and 47.4% of patients with oral and nonrelated multiple cancers ($P < 0.001$).

Association between polymorphisms in alcohol-metabolizing genes and MPT risk

As shown in Table 4, *ADH1B*1* allele carriers had a significantly increased risk of MPTs in the esophagus or upper gastrointestinal tract (OR = 2.093; 95% CI: 1.149–3.812; $P = 0.016$). Similar results were found for *ALDH2*2* allele carriers (OR = 5.186; 95% CI: 2.444–11.004; $P < 0.001$). In addition, the risk of MPTs in the oral cavity was higher for homozygous *ADH1C*1*1* allele carriers (OR = 3.385; 95% CI: 1.433–7.994; $P = 0.005$).

Discussion

In HNCs, patients usually face problems of disease control after treatment, such as local recurrence or distant metastasis. However, some patients develop secondary primary tumors, although the primary index tumor is well-controlled. It has been estimated that about one-third of HNC deaths are attributable to MPTs (20). The site of MPT development varies in HNC, but the most frequent sites are in the head and neck region. The implied mechanism involves "field cancerization," which means that the upper aerodigestive tract receives the same carcinogen exposure in patients with HNC (21–23). However, the survival was poorer in patients with MPTs that occurred in the esophagus or lung in a nationwide study (24). The incidence of multiple primary tumors varies in every patient with HNC (3, 25, 26). Although a regular follow-up CT was arranged after treatment of these patients, some patients with hypopharyngeal and esophageal cancers were still diagnosed in a late tumor stage. Identifying biomarkers for MPT susceptibility in patients with HNC is valuable for an improvement of the detection rate and prolongation of survival. This study provides evidence that carriers of the *ALDH2*2* and *ADH1B*1* alcohol metabolism gene polymorphisms carry a higher risk of MPT occurrence in the esophagus or upper digestive tract. To the best of our knowledge, this study is the first to

Table 3. Distribution of genotypes in alcohol-metabolizing enzyme genes in relation to the occurrence of MPTs

		MPT occurrence [n (%)]				
	Single primary tumor [*n* (%)]	MPTs in oral cavity	MPTs in esophageal or upper GI tract	MPTs in lung or aerodigestive tract	Other MPTs	*P*
ADH1B						
*1*1	65 (13.5)	4 (4.5)	17 (29.8)	1 (9.1)	1(5.3)	<0.001
*1***2**	188 (38.9)	23 (25.8)	23 (40.4)	4 (36.4)	4 (21.1)	
2**2**	230 (47.6)	62 (69.7)	17 (29.8)	6 (54.5)	14 (73.7)	
ADH1C						
1**1**	389 (80.5)	83 (93.3)	43 (75.4)	7 (63.6)	15 (78.9)	0.042
12	84 (17.4)	6 (6.7)	14 (24.6)	4 (36.4)	4 (21.1)	
*2*2	10 (2.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
ALDH2						
*1*1	206 (42.7)	34 (38.2)	9 (15.8)	7 (63.6)	10 (52.6)	<0.001
*1***2**	258 (53.4)	44 (49.4)	47 (82.5)	4 (36.4)	9 (47.4)	
2**2**	19 (3.9)	11 (12.4)	1 (1.8)	0 (0.0)	0 (0.0)	

NOTE: Boldface type indicates the risk (fast-metabolizing or enzyme activity-deficient) allele of alcohol-metabolizing genes.

Table 4. Logistic regression analysis with risk estimates of SNPs in alcohol-metabolizing enzyme genes and the occurrence of MPTs

		Oral cancer with MPTs						
Genotype	Single primary tumor [*n* (%)]	MPTs in oral cavity [*n* (%)]	OR (95% CI)[a]	MPTs in esophageal or upper digestive tract [*n* (%)]	OR (95% CI)[a]	MPTs in lung or aerodigestive tract [*n* (%)]	OR (95% CI)[a]	Other MPTs [*n* (%)]	OR (95% CI)[a]
ADH1B									
***2*2**	230 (47.6)	62 (69.7)	1	17 (29.8)	1	6 (54.5)	1	14 (73.7)	1
*1*1+***1*2**	253 (52.4)	27 (30.3)	0.395 (0.243–0.643)[a]	40 (70.2)	2.093 (1.149–3.812)[b]	5 (45.5)	0.734 (0.219–2.454)	5 (26.3)	0.321 (0.113–0.910)[b]
ADH1C									
***1*2**+*2*2	94 (19.5)	6 (6.7)	1	14 (24.6)	1	4 (36.4)	1	4 (21.1)	1
***1*1**	389 (80.5)	83 (93.3)	3.385 (1.433–7.994)[c]	43 (75.4)	0.757 (0.395–1.450)	7 (63.6)	0.389 (0.110–1.383)	15 (78.9)	0.843 (0.270–2.629)
ALDH2									
*1*1	206 (42.7)	34 (38.2)	1	9 (15.8)	1	7 (63.6)	1	10 (52.6)	1
*1***2**+***2*2**	277 (57.3)	55 (61.8)	1.207 (0.749–1.946)	48 (84.2)	5.186 (2.444–11.004)[c]	4 (36.4)	0.481 (0.132–1.750)	9 (47.4)	0.759 (0.293–1.962)

The OR and 95% CI were calculated using unconditional logistic regression and adjusted for age and alcohol consumption status.
Boldface type indicates the risk (fast-metabolizing or enzyme activity-deficient) allele of alcohol-metabolizing genes.
[a]$P < 0.001$.
[b]$P < 0.05$.
[c]$P < 0.01$.

demonstrate the association between alcohol-metabolizing genes and MPT occurrence in HNC.

ALDH2 plays a key role in alcohol metabolism by catalyzing the conversion of acetaldehyde to acetate. The deficiency allele is *ALDH2*2*, which induces the alcohol flushing syndrome with a higher prevalence in East Asian populations (27). In this study, the prevalence of **2* allele carriers of *ALDH2* among patients with HNC (59.7%) is higher than that in the normal control (49.6%) group (Supplementary Table S1), which is similar to the result reported in Japan (58.9% in patients with cancer vs. 50.9% in controls; ref. 17). In addition, similar to the study of alcohol and HNC risk in Taiwan (28), we found that this *ALDH2* polymorphism significantly increases the HNC risk (Supplementary Table S1). Furthermore, we found that *ALDH2*2* carriers have an increased risk of the occurrence of MPTs in the upper digestive tract (Table 4). ALDH2 deficiency is a well-known risk factor for upper aerodigestive tract cancers, that is, HNC and esophageal cancer (29). The *ALDH2*2* allele encodes a catalytically inactive subunit and causes a high blood level of acetaldehyde, which may be harmful to the upper digestive tract mucosa (30). According to the oral field cancerization concept, oral, pharyngeal, esophageal, and upper aerodigestive tract cancers are exposed to the same carcinogenic environment (22). An interaction between the susceptibility genes and the environment might result in these observations.

Several studies in various populations have found that the esophageal cancer risk in regard to alcohol intake is also closely related to polymorphisms of the *ADH1B* gene (31, 32). In this study, we demonstrated that the *ADH1B*2* allele, with a fast metabolic rate, has a protective effect against HNC risk (Supplementary Table S1). Furthermore, *ADH1B*1*, with a slow metabolic rate, is detrimental to HNC risk. However, when we separated the effect of the *ADH1B* SNP into different subsites, the prevalence of *ADH1B*1* was not different in oral cavity cancer, but the effect was mainly observed in oropharyngeal and hypopharyngeal cancers (Table 2; Supplementary Table S2). Thus, in our study, we demonstrated the necessity of considering subsites in evaluating the effect of SNPs in alcohol-metabolizing genes. Moreover, the *ADH1B*1* allele increased the risk of MPT occurrence in the esophagus and upper digestive tract (Table 4). The protective effect of the *ADH1B*2* allele is consistent with the results of several previous studies in HNC and esophageal cancer (28, 33). Two possible explanations for this consistency are suggested (32): First, the fast initial metabolism may lead to a peak in acetaldehyde exposure, inducing alternative mechanisms to reduce this peak. On the other hand, a more moderate initial metabolism may not induce such a mechanism, resulting in a greater overall exposure. Second, the protective effect of the *ADH1B*2* allele might be due to multiple substrates. ADH1B is involved in retinol metabolism (34); thus, dietary intake of vitamin A with the fast-

metabolizing *ADH1B* allele may protect against upper aerodigestive tract cancers. We observed that the effect of the slow-metabolizing *ADH1B*1* allele was more evident in oropharyngeal and hypopharyngeal cancers (Table 2). The role of *ADH1B*1* in developing MPTs in the oral cavity was protective. In Tsai and colleagues' study, the slow-metabolizing *ADH1B* allele increased the risk of oral cancer in combination with poor dental hygiene. Most of our oral cavity cancer patients received radical surgery during initial treatment. The interaction between *ADH1B*1* and dental hygiene could be influenced by the treatment modality. However, the slow transformation of acetaldehyde by ADH1B increased the exposure to acetaldehyde in the oropharynx and esophagus, which rendered higher risks of MPTs in the upper digestive tract (Table 4).

Several prior studies have suggested that the fast-metabolizing allele of the *ADH1C* gene (*ADH1C*1*) is positively associated with HNC risk or plays no role in modifying the HNC risk (35). In our study, the prevalence of the *ADH1C*1* allele was not different between the controls and patients with HNC (Table 2). However, the *ADH1C*1*1* genotype increased the occurrence of MPTs in the oral cavity (Table 4). A similar result was also observed for the *ADH1B*2*2* genotype (Table 4). Different from the effect on MPTs in the esophagus or upper digestive tract, the fast-metabolizing genotypes of *ADH1B* and *ADH1C* increased the risk of MPTs in the oral cavity. Salivary acetaldehyde concentrations were found to be modulated by the *ADH1C* genotype, with subjects homozygous for the *ADH1C*1* allele having higher salivary acetaldehyde levels than heterozygous subjects (36). Our observations suggest the additive or synergistic effect of salivary and blood acetaldehyde in the oral cavity.

In our study, the SNPs in alcohol-metabolizing enzymes conferred susceptibility to HNC in a Taiwanese population. More interestingly, the *ADH1B*1* allele did not increase the risk of oral cavity cancer. However, our results indicate (Table 2) that the prevalence of *ADH1B*1* increases by tumor subsite from the oropharynx to the tonsil to the hypopharynx. Previous studies analyzed patients with tumors at single subsites (such as the oral cavity, larynx, or esophagus); few studies simultaneously evaluated the SNP effects on different subsites. The second important point in this study is that we demonstrated that patients with susceptibility alleles of alcohol-metabolizing enzyme genes have higher risks of developing multiple primary cancers in the aerodigestive tract. This finding strengthens the possibility of precision preventive medicine. We can incorporate information on genetic susceptibility along with that on environmental exposure. High-risk cancer patients can arrange more frequent imaging exams and pan-endoscopy exams to detect lesions early. This study identifies a group of patients easily overlooked in clinics. Studies on carcinogen-metabolizing enzymes and the risk of MPTs are needed in the future.

This study is the first to investigate the SNPs in alcohol-metabolizing enzyme genes and the risk of multiple primary cancers. In our study, we demonstrated that the *ALDH2*2* allele increased the head and neck cancer risks. The *ADH1B*1* allele significantly increased the risk of oropharyngeal cancer, but not of oral cavity cancer. In addition, the *ALDH2*2* and *ADH1B*1* alleles increased the risk of multiple upper aerodigestive tract cancers.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Novelty and impact statements

SNPs in *ALDH2* and *ADH1B* differ in subsites of head and neck cancers. Patients with **2 ALDH2* and **1 ADH1B* genotypes had ORs of 5.18 and 2.09 in developing MPTs after the diagnosis of index cancer in head and neck. Alcohol-metabolizing enzymes' genetic variations had been linked with susceptibility of MPTs in our study, which has not been addressed before.

Authors' Contributions

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): H.-T. Chien, C.-K. Young, C.-T. Liao, H.-M. Wang, S.-D. Cheng, S.-F. Huang
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): H.-T. Chien, C.-K. Young, C.-T. Liao, S.-F. Huang
Writing, review, and/or revision of the manuscript: H.-T. Chien, C.-K. Young, C.-T. Liao, S.-F. Huang
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): C.-K. Young, T.-P. Chen, S.-F. Huang
Study supervision: S.-F. Huang

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