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Original Article

Impact of multiple *Alcohol Dehydrogenase* gene polymorphisms on risk of laryngeal, esophageal, gastric and colorectal cancers in Chinese Han population

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Abstract: Alcohol intake is positively associated with the risk of upper aerodigestive tract (UADT) cancers; but its effect on gastric or colorectal cancer is controversial. Previous study had identified several single nucleotide polymorphisms (SNPs) of *Alcohol Dehydrogenase* (*ADH*) genes associated with UADT cancers in European and Japanese populations. We sought to determine if these SNPs associated with laryngeal, esophageal, gastric or colorectal cancer in Chinese population. We conducted a case-control study among 1577 cases and 1013 healthy controls from northwest China. Five SNPs associated with UADT cancers risk were selected from previous genome-wide association studies and genotyped using Sequenom Mass-ARRAY technology. Odds ratios and 95% confidence intervals (CIs) were calculated by unconditional logistic regression adjusting for age and gender. We identified that the minor alleles of rs1789924 and rs971074 were associated with decreased risk of laryngeal cancer (OR = 0.311; 95% CI = 0.161-0.602; $P < 0.001$) and esophagus cancer (OR = 0.711; 95% CI = 0.526-0.962; $P = 0.027$) in allelic model analysis, respectively. In the genetic model analysis, we found the "C/T" genotype of rs1789924 was associated with decreased laryngeal cancer risk in codominant model ($P = 0.046$) and overdominant model ($P = 0.013$); the "C/T-T/T" genotype of rs1789924 was associated with reduced risk of laryngeal cancer under dominant model ($P = 0.013$). Additionally, none of the SNPs was associated with gastric or colorectal cancer in our study. Our data shed new light on the association between *ADH* SNPs and respiratory and digestive tract cancers susceptibility in the Han Chinese population.

Keywords: *Alcohol Dehydrogenase* (*ADH*) gene, single nucleotide polymorphism, laryngeal cancer, esophageal cancer, gastric cancer, colorectal cancer

Introduction

Alcohol consumption is one of the most important risk factors for upper aerodigestive tract (UADT) cancers [1, 2]. The metabolism of alcohol releases the carcinogen acetaldehyde as an intermediate [3]. As we know, ethanol is oxidized to acetaldehyde mainly by alcohol dehydrogenase enzymes (ADH), genetic variation in these genes may influence their rate of function, which means *ADH* gene polymorphisms could lead to a relative increase or decrease in exposure to acetaldehyde and this may explain individual differences in UADT cancers susceptibility [4-7].

Several independent genome-wide association scans have identified common *ADH* single-nucleotide polymorphisms (SNPs) associated with risk of UADT cancers. Significant associations between polymorphisms in *ADH1B* and *ADH1C*, and UADT cancers have been observed in European populations [8]. Later, more independent variants *ADH4* (rs3805322), *ADH1B* (rs1229984) and *ADH1C* (rs698 and rs1693482) have been found associated with UADT cancers risk in Japanese population [9]. Additionally, *ADH1B* (rs1229984), *ADH1C* (rs1789924) and *ADH7* (rs971074) have also been associated with UADT cancers risk in two European based multi-centre case-control

Table 1. Primers used for this study

SNP_ID	1st-PCRP	2nd-PCRP	UEP_SEQ
rs3805322	ACGTTGGATGCAAAGCATCTGATCTAGAAC	ACGTTGGATGTCAGGGAGGTTCAGATAAGC	gggtgGATAAGCAGGTTGAGATGTCC
rs1042026	ACGTTGGATGGGGCATTTTATTTGAGTTCC	ACGTTGGATGGGGTAAGGTAAAGGATAGAC	ggaaaATTTACAAGTAGTGAAGGTCC
rs1229984	ACGTTGGATGTTGCCACTAACCACGTGGTC	ACGTTGGATGCTGAATCTGAACAGCTTCTC	cggGGCTGTAGGAATCTGTC
rs1789924	ACGTTGGATGGCTCTCTCTGTTCCACTTTG	ACGTTGGATGTCCCTTTGTCTGCACTAAGC	AATCCACATGGTGCAGTTA
rs971074	ACGTTGGATGATACACTCAGTGGCACCTAC	ACGTTGGATGATGGGCTGTAAGTCAGCTGG	Ggtcagctggtgcatctag

studies [10]. However, few evidence is available concerning these polymorphisms associated with UADT cancer in Chinese populations. What's more, there is evidence on the fact that drinking of alcoholic beverages is causally associated with cancers of the oral cavity, pharynx, larynx and esophagus; but the effect of alcohol consumption on gastric or colorectal cancer is controversial [11]. Therefore, the association between *ADH* gene polymorphisms and gastric or colorectal cancer might be different from that in UADT cancers.

To assess the association between SNPs in alcohol metabolism genes, in particular in *ADH4*, *ADH1B*, *ADH1C* and *ADH7* genes, and UADT, gastric and colorectal cancers. We genotyped five SNPs (rs3805322, rs1042026, rs1229984, rs1789924 and rs971074) of *ADH* genes in a case-control study with 1577 cases (laryngeal cancer in 180, esophageal cancer in 360, gastric cancer in 588, and colorectal cancer in 449) and 1013 controls from northwest China. Our data shed new light on the association between *ADH* SNPs and respiratory and digestive tract cancers susceptibility in the Han Chinese population.

Materials and methods

Study participants

All participants in our study were Han Chinese. A total of 1577 patients newly histologically diagnosed with respiratory and digestive tract cancers (laryngeal cancer in 180, esophageal cancer in 360, gastric cancer in 588, and colorectal cancer in 449) were consecutively recruited between January 2011 and December 2014 at the Xijing Hospital in Xi'an, China. There were no gender, age, or disease-stage restrictions for case recruitment. All cases were previously healthy. Cancers were diagnosed according to the criteria established the International Union Against Cancer tumor-node-metastasis (TNM) classification system (7th ed.) [12].

The controls were 1013 first-visit outpatients at the Xijing Hospital during the same period who were confirmed to have no cancer and no history of neoplasia. Noncancer status was confirmed by medical examinations including radiographic examinations. Those who suspected of having respiratory and digestive tract cancers were examined by physical or endoscopic inspection. Radiographic examinations were carried out for subjects suspected of having cancer after inspection. Controls were selected unrelated and randomly.

Demographic and clinical data

We collected demographic and clinical data via face-to-face interviews using a standardized epidemiological questionnaire, including information on residential regions, age, gender, education status, and family history of cancer. Detailed clinical information on cases was collected from treating physicians or medical chart reviews. All of the participants signed an informed consent agreement. The Human Research Committee for Approval of Research Involving Human Subjects, Xijing Hospital, approved the use of human tissue in this study.

SNP selection and genotyping

We selected five SNPs from previously published polymorphisms associated with UADT cancers [10, 13]. Minor allele frequencies of all SNPs were > 5%, in the HapMap of the Chinese Han Beijing (CHB) population. Extraction of DNA from whole-blood samples was done with GoldMag-Mini Whole Blood Genomic DNA Purification Kits (GoldMag Co., Ltd.; Hainan City, China), and DNA concentration was measured with a NanoDrop 2000 spectrophotometer. The multiplexed SNP MassEXTENDED assay was designed using Sequenom MassARRAY Assay Design 3.0 Software [14]. Genotyping was done with the Sequenom MassARRAY RS1000 system using the standard protocol recommended by the manufacturer. Data management and analysis was done

Table 2. Characteristics of cases and controls in this study

Variables	Laryngeal Cancer			Esophageal Cancer		
	Case (N = 180)	Control (N = 310)	*P*-value	Case (N = 360)	Control (N = 310)	*P*-value
Gender			< 0.001[a]			< 0.001[a]
Male (%)	172 (95.6)	197 (63.5)		288 (80)	197 (63.5)	
Female (%)	8 (4.4)	113 (36.5)		72 (20)	113 (36.5)	
Age						
Mean age ± SD	60.7 ± 10.1	49.4 ± 7.9	< 0.001[b]	60.7 ± 8.9	49.4 ± 7.9	< 0.001[b]
Smoking status			< 0.001[a]			
Nonsmoking (%)	40 (22.2)	210 (69.8)				
Smoking (%)	140 (77.8)	91 (30.2)				
Variables	Gastric Cancer			Colorectal Cancer		
	Case (N = 588)	Control (N = 703)	*P*-value	Case (N = 449)	Control (N = 703)	*P*-value
Gender			< 0.001[a]			0.598[a]
Male (%)	392 (66.7)	396 (56.3)		260 (57.9)	396 (56.3)	
Female (%)	196 (33.3)	307 (43.7)		189 (42.1)	307 (43.7)	
Age						
Mean age ± SD	58.1 ± 11.7	48.6 ± 9.4	< 0.001[c]	59.1 ± 11.8	48.6 ± 9.4	< 0.001[c]

[a]*P* values were calculated from two-sided chi-square tests. [b]*P* values were calculated by Student t tests. [c]*P* values were calculated by Welch's t tests.

using Sequenom Typer 4.0 Software [14, 15]. The corresponding primers used for each SNP in the present study are listed in **Table 1**.

Statistical analysis

We used Microsoft Excel and the SPSS 18.0 statistical package (SPSS, Chicago, IL, USA) to perform statistical analyses. All *P*-values in our study were two-sided, and $P = 0.05$ was considered the cutoff for statistical significance. In all analyses, the lower frequency allele was coded as the 'risk' allele. Control genotype frequencies for each SNP were tested for departure from Hardy-Weinberg equilibrium (HWE) using Fisher's exact test. The χ^2 test was used to compare genotype frequencies in cases and controls [16]. Odds ratios (ORs) [17] and 95% confidence intervals (CIs) were determined using unconditional logistic regression analysis with adjustments for age and gender [18].

Associations between SNPs and risks of respiratory and digestive tract cancers were tested in genetic models by analysis with SNP Stats software, obtained from http://bioinfo.iconcologia.net. Values of OR and 95% CI were calculated as above. Akaike's Information Criterion and Bayesian Information Criterion were applied to choose the best-fit model for each SNP.

Results

A total of 1577 cases and 1013 controls were enrolled in our study. **Table 2** showed the characteristics of cases and controls. There were significant differences in gender and age distribution between the case and control groups ($P < 0.05$) except in colorectal cancer. In addition, smoking was more prevalent in the laryngeal cancer cases than in the matched controls.

Table 3 summarized the major allelic frequency (MAF) of tested SNPs among the individuals in the case and control groups. Two SNPs (rs1042026 in laryngeal and esophageal cancer, rs3805322 in gastric and colorectal cancer) were excluded at the 5% HWE *P*-level. The allelic frequency of other SNPs in the controls group was similar to those of the HapMap Asian population. Through the χ^2 test, we found that rs1789924 (OR = 0.311; 95% CI = 0.161-0.602; $P < 0.001$) was significantly associated with decreasing laryngeal cancer risk. Rs971074 (OR = 0.711; 95% CI = 0.526-0.962; $P = 0.027$) was associated with lessened esophageal cancer risk.

Furthermore, we assumed that the minor allele of each SNP as a risk factor compared with the wild-type allele. Five genetic models (dominant, recessive, additive, codominant, and overdomi-

Table 3. Allele frequencies in cases and controls and odds ratio estimates for Laryngeal, Esophageal, Gastric and Colorectal Cancer

SNP_ID	Gene	Band	Position	Allele A/B[a]	MAF			HWE	Laryngeal Cancer			Esophageal Cancer		
					Laryngeal Cancer Case	Esophageal Cancer Case	Control	*P*-value	ORs	95% CI	*P* value	ORs	95% CI	*P* value
rs3805322	ADH4	4q23	100056998	G/A	0.483	0.462	0.432	0.48	1.229	0.947-1.595	0.12	1.129	0.909-1.402	0.273
rs1042026	ADH1B	4q23	100228466	A/G	0.387	0.279	0.287	0.04[#]	1.567	1.184-2.074	0.002	0.959	0.755-1.217	0.729
rs1229984	ADH1B	4q23	100239319	G/A	0.365	0.355	0.385	0.64	0.917	0.700-1.201	0.529	0.878	0.703-1.097	0.252
rs1789924	ADH1C	4q23	100274286	T/C	0.031	0.077	0.092	0.27	0.311	0.161-0.602	< 0.001*	0.819	0.556-1.207	0.313
rs971074	ADH7	4q23	100341861	A/G	0.128	0.129	0.173	0.37	0.702	0.484-1.020	0.062	0.711	0.526-0.962	0.027*
SNP_ID	Gene	Band	Position	Allele A/B[a]	MAF			HWE	Gastric Cancer			Colorectal Cancer		
					Gastric Cancer Case	Colorectal Cancer Case	Control	*P*-Value	ORs	95% CI	*P* value	ORs	95% CI	*P* value
rs3805322	ADH4	4q23	100056998	G/A	0.440	0.440	0.460	0.03[#]	0.923	0.790-1.079	0.316	0.923	0.780-1.093	0.354
rs1042026	ADH1B	4q23	100228466	A/G	0.279	0.290	0.272	0.45	1.036	0.870-1.232	0.693	1.096	0.910-1.321	0.333
rs1229984	ADH1B	4q23	100239319	G/A	0.368	0.378	0.362	0.17	1.027	0.874-1.206	0.745	1.069	0.899-1.271	0.452
rs1789924	ADH1C	4q23	100274286	T/C	0.106	0.110	0.107	0.84	0.988	0.769-1.270	0.928	1.029	0.787-1.347	0.83
rs971074	ADH7	4q23	100341861	A/G	0.148	0.147	0.154	0.47	0.952	0.767-1.183	0.66	0.947	0.748-1.198	0.649

[a]Minor allele; [#]site with HWE $P \leq 0.05$ is excluded; *P value ≤ 0.05 indicates statistical significance; HWE, Hardy-Weinberg Equilibrium; ORs, odds ratios; CI: confidence interval.

Table 4. Genotypic model analysis of relationship between rs1789924 and laryngeal cancer (adjusted by gender, age and smoking status)

Model	Genotype	Control	Case	OR (95% CI)	*P*-value	AIC	BIC
Codominant	C/C	245 (82.5%)	168 (93.8%)	1	0.046*	410.9	435.9
	C/T	51 (17.2%)	11 (6.2%)	0.38 (0.17-0.84)			
	T/T	1 (0.3%)	0 (0%)	0.00 (0.00-NA)			
Dominant	C/C	245 (82.5%)	168 (93.8%)	1	0.013*	408.9	429.8
	C/T-T/T	52 (17.5%)	11 (6.2%)	0.37 (0.17-0.84)			
Recessive	C/C-C/T	296 (99.7%)	179 (100%)	1	0.87	415.1	435.9
	T/T	1 (0.3%)	0 (0%)	0.00 (0.00-NA)			
Overdominant	C/C-T/T	246 (82.8%)	168 (93.8%)	1	0.013*	409	429.8
	C/T	51 (17.2%)	11 (6.2%)	0.38 (0.17-0.84)			
Log-additive	---	---	---	0.37 (0.17-0.84)	0.013*	408.9	429.8

ORs, odds ratios; CI: confidence interval; AIC: Akaike's Information criterion; BIC: Bayesian Information criterion. *P value ≤ 0.05 indicates statistical significance.

Table 5. Genotypic model analysis of relationship between rs971074 and Esophageal cancer (adjusted by gender and age)

Model	Genotype	Control	Case	OR (95% CI)	*P*-value	AIC	BIC
Codominant	G/G	208 (68%)	269 (75.6%)	1	0.25	699.4	721.8
	A/G	91 (29.7%)	82 (23%)	0.76 (0.50-1.16)			
	A/A	7 (2.3%)	5 (1.4%)	0.45 (0.12-1.75)			
Dominant	G/G	208 (68%)	269 (75.6%)	1	0.14	697.9	715.9
	A/G-A/A	98 (32%)	87 (24.4%)	0.73 (0.49-1.10)			
Recessive	G/G-A/G	299 (97.7%)	351 (98.6%)	1	0.29	699	717
	A/A	7 (2.3%)	5 (1.4%)	0.49 (0.13-1.87)			
Overdominant	G/G-A/A	215 (70.3%)	274 (77%)	1	0.23	698.7	716.7
	A/G	91 (29.7%)	82 (23%)	0.78 (0.51-1.18)			
Log-additive	---	---	---	0.74 (0.51-1.06)	0.1	697.5	715.4

ORs, odds ratios; CI: confidence interval; AIC: Akaike's Information criterion; BIC: Bayesian Information criterion.

nant) were applied to analyze the associations between the SNPs and laryngeal, esophageal, gastric and colorectal cancers risk using a logistic regression test. We found that the "C/T" genotype of rs1789924 was associated with decreased laryngeal cancer risk, based on the codominant model (OR = 0.38; 95% CI = 0.17-0.84; P = 0.046) and overdominant model (OR = 0.38; 95% CI = 0.17-0.84; P = 0.013). Additionally, the "C/T-T/T" genotype of rs1789924 associated with reduced risk of laryngeal cancer as revealed by the dominant model (OR = 0.37, 95% CI = 0.17-0.84, P = 0.013) (**Table 4**). However, we had not identified any associations between rs971074 and esophageal cancer risk after adjusting by gender and age (**Table 5**).

Discussion

In the present case-control study, we investigated the association between five SNPs (rs3805322, rs1042026, rs1229984, rs1789924 and rs971074) of *ADH* genes and risk of laryngeal, esophageal, gastric and colorectal cancers in Chinese Han population. Among these SNPs, rs1789924 in *ADH1C* was found to be significantly associated with decreasing laryngeal cancer risk, rs971074 in *ADH7* was associated with lessened esophageal cancer risk. Additionally, the "C/T" and "C/T-T/T" genotype of rs1789924 was associated with reduced risk of laryngeal cancer in genetic model analysis. However, none of these SNPs was found to be associated with risk of gastric or colorectal cancer.

Rs1789924 is located at 5' near gene of *ADH1C*, and predicted to affect the binding of transcription factor [19]. To the best of our knowledge, this is the first study to examine the association between rs1789924 and laryngeal cancer risk. One study showed that rs1789924

was related to lessened drinking behavior [20]. In our study, we found that rs1789924 was a protective variant for laryngeal cancer. It could be hypothesized that rs1789924 may play a role in preventing the apoptotic response of laryngeal cells that are exposed to genotoxic stress caused by alcohol-related carcinogens. However, we failed to replicate rs1789924 association with esophageal cancer that identified in previous study [19]. Therefore, these findings need further large and more ethnically diverse population-based studies to validate.

The SNP rs971074 positioned in the *ADH7* locus on chromosome 4q23, was significantly decreased the risk of UADT cancers in previous GWAS study in European populations [10]. Our data showed its association with decreasing esophageal cancer, but it was not significant any more after adjusting by age and sex. The inconsistent association findings might be attributed to the different ethnicity of the subjects enrolled in each study.

Among the remaining three SNPs (rs3805322, rs1042026 and rs1229984), rs3805322 was relatively less studied. Rs3805322 was associated with UADT cancers in Japanese population [9], while it's not significant in our study population. The SNP rs1042026 were found to be associated with UADT cancers risk in several studies. However, the past results were inconsistent [21-23], it's also not significant in our study. Previous studies had shown that rs1229984 was associated with UADT cancers but not significant in colorectal cancer in European [24, 25], and we also didn't detect any associations between rs1229984 and gastric or colorectal cancer in Chinese.

Despite the current study possessing enough power, some limitations should be considered. As we know, respiratory and digestive tract cancers are all very heterogeneous diseases and alcohol drinking is an important risk factor for them. Because the sample size of our study was relatively small and the data of alcohol intake was absent, we could not explore how genetic polymorphisms and alcohol consumption interact in these cancers. So the association between *ADH* gene polymorphism and drinking status and clinical subtype need to be evaluated in future studies.

In conclusion, our study has described the association between rs1789924 (*ADH1C*) and laryngeal cancer risk in Chinese Han population. Although we do not find any significant results, this is the first report on the association between *ADH* gene polymorphisms (rs3805322, rs1042026, rs1229984, rs1789924 and rs971074) and four kinds of respiratory and digestive tract cancers (laryngeal, esophageal, gastric and colorectal cancer) simultaneously in Chinese population.

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Disclosure of conflict of interest

None.

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