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RAPID COMMUNICATION

Genetic polymorphisms of *ADH2* and *ALDH2* association with esophageal cancer risk in southwest China

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Abstract

AIM: To evaluate the impact of alcohol dehydrogenase *2 (ADH2)* and aldehyde dehydrogenase *2 (ALDH2)* polymorphisms on esophageal cancer risk.

METHODS: One hundred and ninety-one esophageal cancer patients and 198 healthy controls from Yanting County were enrolled in this study. *ADH2* and *ALDH2* genotypes were examined by polymerase-chain-reaction with the confronting-two-pair-primer (PCR-CTPP) method. Unconditional logistic regression was used to calculate the odds ratios (OR) and 95% confidence interval (95% CI).

RESULTS: Both *ADH2*1* allele and *ALDH2*1/*2* allele showed an increased risk of developing esophageal cancer. The adjusted OR (95% CI) for *ADH2*1* allele compared with *ADH2*2/*2* was 1.65 (95% CI = 1.02-2.68) and 1.67 (95% CI = 1.02-2.72) for *ALDH2*1/*2* compared with *ALDH2*1/*1*. A significant interaction between *ALDH2* and drinking was detected regarding esophageal cancer risk, the OR was 1.83 (95% CI = 1.13-2.95). Furthermore, when compared with *ADH2*2/*2* and *ALDH2*1/*1* carriers, *ADH2*1* and *ALDH2*2* carriers showed an elevated risk of developing esophageal cancer among non-alcohol drinkers (OR = 2.46, 95% CI = 0.98-6.14), and a significantly elevated risk of developing esophageal cancer among alcohol drinkers among alcohol drinkers (OR = 9.86, 95% CI = 3.10-31.38).

CONCLUSION: *ADH2* and *ALDH2* genotypes are associated with esophageal cancer risk. *ADH2*1* allele and *ALDH2*2* allele carriers have a much higher risk of developing esophageal cancer, especially among alcohol drinkers.

Key words: Esophageal cancer; Alcohol dehydrogenase 2; Aldehyde dehydrogenase 2; Genetic polymorphisms

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INTRODUCTION

Epidemiological studies have consistently shown that alcohol drinking is a strong risk factor for esophageal cancer[1-3]. Alcohol is not a carcinogen, but its primary metabolite, acetaldehyde, has been proven carcinogenic in experimental models[4,5]. When consumed through drinking, ethanol is metabolized primarily by class I alcohol dehydrogenase (ADH2) into acetaldehyde, an intermediate metabolite, followed by aldehyde dehydrogenase (ALDH2) into acetic acid in humans[6]. Acetaldehyde, a well-known carcinogen in animals, plays an important role in alcohol toxicity to humans[7]. The encoding genes of the two representative alcohol-metabolizing enzymes display polymorphisms which may modulate individual differences in alcohol-oxidizing capacity and drinking behavior[7,8]. *ADH2*2/*2* has about 40 times higher Vmax than the less-active *ADH2*1/*1*. *ALDH2*2* allele encodes a catalytically inactive subunit for the ALDH2 polymorphism[6,9]. Individuals with the *ALDH2*1/*2* genotype have only 6.25% of normal *ALDH2 *1/*1* activity, indicating a dominant effect of *ALDH2*2*[10]. *ADH2*2* allele and *ALDH2*2* allele, both leading to high acetaldehyde concentrations, are clustered in East Asian populations[6,11,12]. Therefore, polymorphisms of these two genes may exert their effects on esophageal cancer susceptibility. Although several studies on *ADH2* or *ALDH2* polymorphisms and esophageal cancer risk have been conducted to clarify their association[13-15], investigations on non-alcoholic drinkers are limited[16-19].

Yanting, a rural county of Sichuan Province, is one of the areas with the highest esophageal cancer mortality in China[20]. According to the report from Tumor Registry of China, the average incidence rate in this area was $100.5/10^5$ for males and $76.5/10^5$ for females during 1999-2003, which was higher than that in Linxian County and lower than that in Cixian County of Hebei Province, China. Our previous study in Yanting County has shown that alcohol drinking and smoking are common in Yanting County and the main contributors to esophageal cancer[21]. To further study alcohol-related gene polymorphisms and gene-environment interaction on esophageal cancer, a case-control study was conducted in Yanting County.

MATERIALS AND METHODS

Subjects

Esophageal cancer patients were consecutively collected from the Hospital of Yangting Cancer Research Institute (YCRI) from July 2003 to July 2004. All the patients having lived in Yanting County for more than five years were histologically diagnosed as esophageal cancer within 6 mo at the age of 35-85 years. A total of 191 patients (183 with squamous cell carcinoma and 8 with adenocarcinoma) were recruited for the study. One hundred and ninety-one healthy residents from Yanting County served as controls. In total, 191 patients and 198 controls completed a questionnaire and each provided 1 mL blood. The questionnaire included basic demographic data, information on esophageal cancer and habits such as smoking and alcohol drinking, as well as information on food and nutrition.

The ethics committee of each collaborating institution reviewed and approved the study, and informed consent was obtained from all participants.

Genotyping of ALDH2 and ADH2

Genotyping was based upon duplex polymerase-chain-reaction with the confronting-two-pair-primer (PCR-CTPP) method[7]. Briefly, the sequences of four primers used for ADH2 polymorphism are F1 ADH2: 5'-GGG CTTTAGACTGAATAACCTTGG-3'; R1 ADH2: 5'-AAC CACGTGGTCATCTGTGC-3'; F2 ADH2: 5'-GGTGGC TGTAGGAATCTGTCA-5'; R2 ADH2: 5'-AGGGAA AGAGGAAACTCCTGAA-3'. The sequences of primers used for ALDH2 polymorphism are F1 ALDH2: 5'-TGC TATGATGTGTTTGGAGCC-3'; R1 ALDH2: 5'-CCC ACACTCACAGTTTTCACTTC-3'; F2 ALDH2: 5'-GGG CTGCAGGCATACACTA-3'; R2 ALDH2: 5'-GGC TCCGAGCCACCA-3'. Each 25 μL reaction mixture contained 1.3 U Tag biocatalysts, 1.8 mmol/L Mg^{2+}, 0.24 mmol/L dNTPs, 8 primers, 15 pmol of each primer and 5-8 μL template. The PCR conditions were as follows: initial denaturation at 94℃ for 5 min, followed by 35 cycles at 94℃ for 65 s, at 60℃ for 65 s, at 72℃ for 90 s, and a final extension at 72℃ for 5 min. After transient centrifugation, agarose electrophoresis was conducted. The PCR products included 119 bp fragments of *ALDH2*1* allele, 98 bp fragments of *ALDH2*2* allele, 219 bp fragments of *ADH2* and *ADH2*1* allele, 280bp fragments of *ADH2*2* allele. The 176 bp and 219 bp fragments were the common fragments of the two alleles.

Table 1 Characteristics of patients and controls *n* (%)

Characteristic	Patients *n* = 191	Controls *n* = 198	*P*[1]	OR (95% CI)[2]
Age (yr)				
< 50	28 (14.7)	84 (42.9)		
50-64	118 (61.7)	67 (33.4)	*P* < 0.001	-
⩾ 65	45 (23.6)	47 (23.7)		
Mean age (SD)	58.3 (8.3)	52.8 (13.2)	-	-
Sex				
Male	126 (66.0)	122 (61.6)	*P* = 0.372	-
Female	65 (34.0)	76 (38.4)		-
Smoking status				
Non-smokers[3]	75 (39.3)	121 (61.1)	*P* < 0.001	1.00 (References)
Current smokers	116 (60.7)	77 (38.9)		3.76 (2.01-6.73)
Alcohol drinking status				
Non-drinkers[4]	80 (41.9)	128 (64.7)		1.00 (References)
Current alcohol drinkers	111 (58.1)	70 (35.3)	*P* < 0.001	3.16 (1.91-5.24)

[1]*P* value by chi-square test; [2]ORs for smoking and drinking were adjusted for age and sex, rapid food eating, quality of drinking water, consumption of fresh fruits, vegetable and eggs; [3]Non-smokers including ex-smokers; [4]Non-drinkers including ex-drinkers.

Statistical analysis

Statistical analyses were performed using the STATA statistical package (version 8, STATA, College Station, TX). Demographic data, smoking and drinking status were compared between patients and controls by chi-square test. The subjects smoking more than 10 cigarettes per week for at least 6 mo were defined as current smokers. The subjects consuming more than 50 mL of distilled spirits per week for at least 6 mo were defined as current drinkers. The odds ratio (OR) and 95% confidence interval (95% CI) generated in unconditional logistic regression model were used as measures of association for the risk of esophageal cancer. The relationship of ALDH2 and ADH2 polymorphisms with esophageal cancer risk was determined after adjustment for sex, age, smoking, rapid food eating, quality of drinking water, consumption of fresh fruits, vegetables and eggs. The combined effect of alcohol consumption and *ADH2* and *ALDH2* genotypes on esophageal cancer was also examined in this study. Chi-square test was used to check the Hardy-Weinberg equilibrium (HWE) in controls for the assessment of discrepancies between genotype and allele frequencies.

RESULTS

The characteristics of subjects are listed in Table 1. The mean age of 191 patients and 198 controls was 58.3 and 52.8 years, respectively. There was a significant difference in smoking and alcohol drinking status ($P < 0.001$) between patients and controls. When compared with non-smokers, the adjusted OR of current smokers was 3.76 (95% CI = 2.01-6.73). Current alcohol drinkers also showed an increased risk of developing esophageal cancer (OR = 3.16, 95% CI = 1.91-5.24) when compared with non-drinkers. Almost all the alcohol drinkers drank hard

Table 2 *ADH2, ALDH2* **genotype and allele frequencies and ORs for esophageal cancer** *n* **(%)**

ADH2	Cases (*n* = 191)	Controls (*n* = 198)	OR (95% CI)[1]
**2/*2*	78 (40.8)	100 (50.5)	1.00 (Reference)
**1/*2*	80 (41.9)	76 (38.4)	1.89 (1.10-3.22)
**1/*1*	33 (17.3)	22 (11.1)	1.91 (0.92-3.95)
**1/*2 + *1/*1*	113 (59.2)	98 (49.5)	1.65 (1.02-2.68)
ALDH2			
**1/*1*	90 (47.1)	108 (54.5)	1.00 (References)
**1/*2*	98 (51.3)	76 (38.4)	1.67 (1.02-2.72)
**2/*2*	3 (1.6)	14 (7.1)	0.26 (0.06-1.09)
**1/*2 + *2/*2*	101 (52.9)	90 (45.5)	1.43 (0.89-2.30)
Allele frequencies			OR (95% CI)[2]
ADH2			
**2*	236 (61.8)	276 (69.7)	1.00 (References)
**1*	146 (38.2)	120 (30.3)	1.42 (1.06-1.92)
ALDH2			
**1*	278 (72.8)	292 (73.7)	1.00 (References)
**2*	104 (27.2)	104 (26.3)	1.05 (0.76-1.44)

[1]ORs for gene frequencies were adjusted for sex, age, smoking, drinking, rapid food eating, quality of drinking water, consumption of fresh fruits, vegetable and eggs; [2]ORs for allele frequencies were not adjusted.

liquor containing over 48% ethanol in this area and the main tobacco type was cigarette (data not shown).

The genotype and allele distribution of *ADH2* and *ALDH2* and their OR (95% CI) for esophageal cancer risk are listed in Table 2. The ADH2 genotype frequency was 50.5% for *ADH2*2/*2*, 38.4% for *ADH2*1/*2*, and 11.1% for *ADH2*1/*1* in controls, which were in accordance with the HWE (P = 0.20) (data not shown). When compared with the *ADH2*2/*2* genotype, the esophageal cancer risk in subjects harboring *ADH2*1* allele was significantly elevated (OR = 1.65, 95% CI = 1.02-2.68). The OR for *ADH2*1* allele carriers was 1.42 (95% CI = 1.06-1.92) when compared with *ADH2*2* allele carriers. The frequency of *ALDH2*1/*1*, *ALDH2*1/*2* and *ALDH2*2/*2* was 54.6%, 38.4% and 7.1%, respectively, in controls, which were also in accordance with the HWE (P = 0.90) (data not shown). The *ALDH2*1/*2* genotype was associated with an increased risk of developing esophageal cancer (OR = 1.67, 95% CI = 1.02-2.72).

The combined effect of alcohol consumption and *ADH2* and *ALDH2* genotypes on esophageal cancer risk is shown in Table 3. When compared with non-drinkers harboring *ADH2*2/*2* genotype, alcohol drinkers carrying *ADH2*1* showed an increased risk of developing esophageal cancer (OR = 3.94, 95% CI = 1.76-8.81). Similarly, a significantly increased risk of developing esophageal cancer was found in alcohol drinkers harboring *ALDH2*2* genotype (OR = 4.82, 95% CI = 2.06-11.27), compared with non-drinkers harboring *ALDH2*1/*1* genotype. Furthermore, a significant interaction between *ALDH2* and alcohol drinking was detected regarding esophageal cancer risk (adjusted OR = 1.83, 95% CI = 1.13-2.95) (data not shown). When compared with *ADH2*2/*2* and *ALDH2*1/*1* carriers, *ADH2*1* and *ALDH2*2* carriers showed an elevated risk of developing esophageal cancer in non-drinkers (OR = 2.46, 95% CI = 0.98-6.14) and a significantly elevated risk of developing esophageal cancer in alcohol drinkers (OR = 9.86, 95% CI = 3.10-31.38).

DISCUSSION

In the present study, the risk of developing esophageal cancer was significantly increased in *ALDH2*1/*2* gene carriers; subjects with *ADH2*1* allele had a higher risk of developing esophageal cancer than those with *ADH2*2/*2*; carrying both *ALDH2*2* allele and *ADH2*1* allele, suggesting that alcohol drinking greatly increases the susceptibility to esophageal cancer.

*ALDH2*2* allele encoding an inactive subunit of ALDH2 is prevalent in Asian[22]. It was reported that acetaldehyde concentrations after drinking alcohol are mainly dependent on the enzyme activation of *ALDH2*[6,23]. After consumption of alcohol, blood acetaldehyde concentrations in those with *ALDH2*2/*2* and *ALDH2*1/*2* are 19- and 6-fold higher than in those with *ALDH2*1/*1*[24]. Case-control studies of Japanese and Chinese alcohol drinkers[10,15,16,17,19,25,26] consistently demonstrated that inactive *ALDH2*1/*2* is a strong risk factor for esophageal cancer. Our data also show that individuals with *ALDH2*1/*2* (OR = 1.67, 95% CI = 1.02-2.72) had a significantly increased risk of developing esophageal cancer compared to those with *ALDH2*1/*1*. Furthermore, our results reveal that there was a significant interaction between *ALDH2* and alcohol drinking, indicating that esophageal cancer is associated with alcohol drinking, which is influenced by the polymorphism of *ALDH2*.

Previous case-control studies investigating the association between *ADH2* genotype and esophageal cancer demonstrated that *ADH2*1* allele independently enhances esophageal cancer risk[6,10,14,17,19]. In our study, the adjusted OR for subjects carrying *ADH2*1* allele was 1.65 (95% CI = 1.02-2.68), which is in line with former studies[6,10,14,17,19]. There are several reasons which may explain this finding. ADH2 is the predominant enzyme among low-Km class I ADHs expressed in the esophagus[27]. In *ADH2*1/*1* homozygotes, concentrations of ethanol may linger in the esophageal mucosa during the slow oxidation of *ADH2*. Although ethanol is not a cancerogen itself, tobaccos and some other exogenous cancerogens would be assimilated much easier, thus increasing the effect of cancerogen. Besides, ethanol can induce the composition of phase I drug metabolism enzymes such as CYP2E1. Moreover, alcohol drinkers with *ADH2*1* genotype tend to have experienced 'binge-drinking' and withdrawal syndrome earlier in life than those with other genotypes[14,23]. Therefore, *ADH2*1*-mediated alcohol-related events may contribute to the enhancement of esophageal cancer risk in alcohol drinkers.

It was reported that combination of *ADH2*1* allele and *ALDH2*2* allele can greatly enhance cancer risk among alcoholics[10] and general populations[6,19,23]. Carrying these two alleles simultaneously indicates a longer time of exposure to alcohol and highly-concentrated acetaldehyde, thus increasing the individual's susceptibility to esophageal cancer. In the present study, the OR for alcohol drinkers

Table 3 Combined effect of alcohol consumption and *ADH2* and *ALDH2* polymorphisms on esophageal cancer *n* (%)

ADH2		Alcohol drinking status					
		Non-drinkers[2]			Current alcohol drinkers		
		Cases (*n* = 80)	Controls (*n* = 128)	OR (95% CI)[1]	Cases (*n* = 111)	Controls (*n* = 70)	OR (95% CI)[1]
*2/*2		37 (46.3)	65 (50.8)	1.00 (References)	41 (36.9)	35 (50.0)	1.88 (0.86-4.15)
*1/*2 or *1/*1		43 (53.7)	63 (49.2)	1.21 (0.63-2.33)	70 (63.1)	35 (50.0)	3.94 (1.76-8.81)
ALDH2							
*1/*1		33 (41.3)	67 (52.3)	1.00 (References)	57 (51.4)	41 (58.6)	3.15 (1.39-7.13)
*1/*2 or *2/*2		47 (58.7)	61 (47.7)	2.03 (1.03-3.99)	54 (48.6)	29 (41.4)	4.82 (2.06-11.27)
ADH2 and ALDH2							
*2/*2	*1/*1	15 (18.8)	42 (32.8)	1.00 (References)	17 (15.3)	20 (28.6)	2.54 (0.84-7.67)
*1/*2 or *1/*1	*1/*2 or *2/*2	25 (31.3)	38 (29.7)	2.46 (0.98-6.14)	30 (27.0)	14 (20.0)	9.86 (3.10-31.38)

[1]ORs were adjusted for sex, age, smoking, rapid food eating, quality of drinking water, consumption of picked vegetables and fresh fruits, vegetables and eggs; [2]Non-drinkers including ex-drinkers.

with both *ADH2*1* allele and *ALDH2*2* allele was 9.86 (95% CI = 3.10-31.38), which is consistent with former studies[10].

Some limitations of this study should be considered. One is that controls were selected from residents in Yanting County and their basic features are consistent with general people, such as smoking and alcohol drinking. In this study, the genotype distribution among the controls closely conformed to the Hardy-Weinberg equilibrium. So our control group represents the general population of Yanting County. In addition, the present study was not an age matched case control study and age is a risk factor for esophageal cancer. However, our results are age-adjusted and may not be biased by age. The small number of subjects is another limitation, so further studies in a larger scale appear warranted.

In conclusion, *ADH2* and *ALDH2* genotypes are associated with esophageal cancer risk. In addition, the risk of developing esophageal cancer increases in subjects carrying *ADH2*1* allele and *ALDH2*1* allele, especially in alcohol drinkers. Our present findings provide more information on the *ADH2* and *ALDH2* polymorphisms of esophageal cancer in Chinese.

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COMMENTS

Background

Epidemiological studies have consistently shown that alcohol drinking is a strong risk factor for esophageal cancer. When consumed through drinking, ethanol is metabolized primarily by class I alcohol dehydrogenase (ADH2) into acetaldehyde, an intermediate metabolite, followed by aldehyde dehydrogenase (ALDH2) into acetic acid in humans. Acetaldehyde, a well-known carcinogen in animals, plays an important role in alcohol toxicity to humans. *ADH2*2* allele and *ALDH2*2* allele, both leading to high acetaldehyde concentrations, are clustered in East Asian populations. Therefore, the polymorphism of these two genes may exert effect on esophageal cancer susceptibility.

Research frontiers

Esophageal cancer risk is associated with habits and food consumption, such as smoking, alcohol drinking, consumption of fresh fruits and vegetables. However, the effect of gene polymorphisms or gene-environment interaction on esophageal cancer risk has become a hotspot in recent researches. The present study reports the association of ADH2 and ALDH2 gene polymorphisms with esophageal cancer risk. Furthermore, interaction and combination impacts on esophageal cancer risk between gene polymorphisms and alcohol drinking are also analyzed and discussed.

Innovations and breakthroughs

Our study showed that *ADH2* and *ALDH2* polymorphisms were associated with esophageal cancer risk in a high-incidence area of southwest China. Previous studies were mainly conducted in Japanese males or alcoholics. In addition, our controls were collected from the healthy residents and the patients were histologically diagnosed as esophageal cancer within 6 mo, which is superior to hospital-based case-control studies.

Applications

The present study indicates ADH2 and ALDH2 genotypes are associated with esophageal cancer risk, the risk of developing esophageal cancer increases in subjects carrying ADH2*1 allele and ALDH2*2 allele, especially in alcohol drinkers. The present findings can provide more information on the ADH2 and ALDH2 polymorphisms of esophageal cancer in Chinese and help make the prevention strategy against esophageal cancer in China.

Terminology

Alcohol dehydrogenase 2 (*ADH2*): A zinc-containing enzyme which oxidizes primary and secondary alcohols or hemiacetals in the presence of NAD. In alcoholic fermentation, it catalyzes the final step of reducing aldehyde to alcohol in the presence of NADH and hydrogen. Dehydrogenase 2 (*ALDH2*): An enzyme that oxidizes aldehyde in the presence of NAD+ and water to acid and NADH. Genetic polymorphisms: The regular and simultaneous occurrence of two or more discontinuous genotypes in a single interbreeding population. The concept includes differences in genotypes ranging in size from a single nucleotide site to large nucleotide sequences visible at a chromosomal level.

Peer review

This is an interesting paper investigating the association between ADH2 and ALDH2 polymorphisms, environmental factors and esophageal cancer risk in a relatively small cohort of cancer patients. The main conclusion of the manuscript is that ADH2 and ALDH2 genotypes are associated with esophageal cancer risk; the risk of developing esophageal cancer increases greatly in subjects carrying ADH2*1 allele and ALDH2*2 allele, especially in alcohol drinkers.

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