RESEARCH COMMUNICATION

Esophageal Squamous Cell Carcinoma and ALDH2 and ADH1B Polymorphisms in Chinese Females

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Abstract

Aim: Alcohol dehydrogenase-IB (ADH1B) and aldehyde dehydrogenase-2 (ALDH2) are the key enzymes for elimination of ethanol and acetaldehyde, the latter being an established animal carcinogen produced after drinking. In this study, we aimed to evaluate the contribution of ADH1B and ALDH2 polymorphisms to the risk of esophageal squamous cell (ESCC) in Chinese females. Methods: A total of 81 pathologically-proven female ESCC cases and 162 female controls were recruited from the Affiliated Hospital of Medical College of Armed Police Forces of PRC in China. ADH1B and ALDH2 polymorphisms were genotyped using PCR-CTPP. Results: Compared with those with ADH1B*2/*2, individuals with ADH2*1/*2 and ADH2*1/*1 had 1.47 and 2.36-fold, respectively, increased risk of developing ESCC (95%CI=0.84-2.58, 95%CI=1.14-5.79) after adjusting for alcohol consumption and other covariates. Significantly increased risk was also noted among subjects with ALDH2*1/*2 (adjusted OR=3.24, 95%CI=1.45-5.36), when compared to those with ALDH2*1/*1. Risk was greater in heavy drinking females carrying ADH1B *1/*1 or ALDH2*1/*2 genotypes compared to those with ADH1B*2 and ALDH2*1/*1. Moreover, we found a significant trend of ESCC risk with alcoholic consumption in women with ALDH2*1/*2. Conclusion: Chinese women with ADH1B *1/*1 or ALDH2*1/*2 have elevated risk of ESCC similarly to men. Women with inactive ADH1B and ALDH2 should reduce drinking and increase their intake of vegetable and fruit to prevent development of esophageal cancer.

Keywords: ADH1B - ALDH2 - gene polymorphisms - esophageal squamous cell carcinoma

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Introduction

Epidemiologic studies have demonstrated that drinking alcoholic beverages is associated with the development of cancers in oral cavity, pharynx, larynx, and esophagus (IARC, 1988). The ethanol in alcohol beverages and the acetaldehyde associated with alcohol consumption have recently been classified as Group 1 human carcinogens by the World Health Organization (WHO) and International Agency For Research On Cancer (IARC)(Baan et al., 2007; Secretan et al., 2009). The ethanol consumed in alcohol beverages is primarily metabolized by alcohol dehydrogenases (ADHs), including ADH1B (previously called ADH2), to acetaldehyde, and then to acetic acid, mainly by low-Km aldehyde dehydrogenase-2 (ALDH2) (Yokoyama and Omori, 2005).

Genetic variation in the ability to metabolize alcohol might be associated with esophageal cancer risk. Thehomodimer of ADH1B encoded by ADH1B*1/*1 has only 1/100 and 1/200 of the ethanol oxidizing capacity of the isozymes encoded by ADH1B*1/*2 and ADH1B*2/*2, respectively, and ADH1B*1/*1 genotype carriers experience prolonged exposure to ethanol after heavy drinking(Yin et al., 1984). The mutant ALDH2*2 allele is also prevalent in east Asians and encodes a catalytically inactive subunit. ALDH2*2 allele carriers experience unpleasant flushing responses after drinking a small amount of ethanol because of severe acetaldehydemia(Harada et al., 1981). Several studies in Asian countries revealed that ADH1B*1 and ALDH2*2 allele as a strong positive factor and a strong negative risk factor of esophageal cancer(Higuchi et al., 1995; Chen et al., 1999; Yokoyama and Omori, 2005). However, most of the researches in this field are focused on males, otherwise, only a small number of female esophageal cancer patients included in previous study (Yang et al., 2010). The incidence of esophageal cancer has a male to female ratio of up to 8:1(IARC, 2011). The age-adjusted mortality rate for esophageal cancer per 100,000 Chinese men in 2008 was estimated to be 22.9, whereas that for Chinese women was 8.2 (IARC, 2011). The difference incidence of esophageal cancer of gender suggested the differences in drinking, smoking, dietary habit and genetics.

Previous studies in Japanese and Chinese have provided conflicting results in regard to the relationship

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between alcohol drinking and esophageal cancer in women (Tuyns, 1983; Castellsague et al., 1999; Gallus et al., 2001; Engel et al., 2003). Several studies revealed that women have a lower risk of esophageal cancer than men due to lower consumption of alcohol. But if taking the similar consumption of alcohol, the cancer risk is more high in women (Negri et al., 1992; Engel et al., 2003). A previous meta-analysis showed that women may be more prone to esophageal cancer than men if taking the same dose of alcohol (Bagnardi et al., 2001). Therefore, if the alcohol drinking plays a more significant role in esophageal cancer risk in women, the ALDH2 and ADH1B genotypes may affect the risk of esophageal cancer in women. A meta-analysis reported ADH1B*1/*1 and ALDH2*1/*2 were associated with a higher risk among heavy drinking women than men, but this study only included two studies conducted in Japan.

Although incidence of esophageal cancer in China is among the highest in the world, the evidence of the effect of ALDH2 and ADH1B genotypes on ESCC risk is lacking. We therefore conducted a case-control study in China to investigate the effect of the two genotypes on ESCC risk in women in a Chinese population.

Materials and Methods

Esophageal cancer patients were consecutively collected from the affiliated hospital of the Armed Police College of Medicine from June 2009 to December 2010. All Chinese female patients with newly diagnosed primary esophageal cancer in the hospital were invited for face-to-face interviews within one months after diagnosis. All cases recruited in our study were histologically confirmed. Among a total of 84 eligible cases, 81 were interviewed with a participation rate of 96%. 162 controls were randomly selected from people who requested general health examinations in the same hospital during the same period and were confirmed to have no malignancy, digestive diseases, chronic diseases and also no prior history of malignancy. The controls were matched with cases by ages within five years age.

Genotyping of ALDH2 and ADH2

Genotyping was based upon duplex polymerase-chain-reaction with the confronting-two-pairprimer (PCR-CTPP) method(Tamakoshi et al., 2003). Briefly, the sequences of primers used for ADH1B polymorphisms are 5'-ATTCTGTAGATGGTGGCTGT-3' and 5'-GAAGGGGGGTCACCAGGTTG-3'. The sequences of primers used for ALDH2 polymorphism are 5'-CCCTTTGGTGGCTAGAAGATG-3' and 5'-CCACACTCACAGTTTTCTCTT-3'. Each 25 μL reaction mixture contained 1.3 U Tag biocatalysts, 1.8 mmol/L Mg2+, 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°C for 2 min, followed by 40 cycles at 94°C for 65 s, at 60°C for 60 s, at 72°C for 50 s, and a final extension at 72°C for 10 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

Statistical analysis

All analysis was performed by using the STATA statistical package (version 9, STATA, College Station, TX). Demographic data, smoking, drinking status, dietary habits and lifestyles were collected. Alcohol drinking was categorized into former, never and current drinking. Individuals who quit drinking more than one year were considered as former drinkers, and individuals who drank more than 200ml beers, 125 wine ml and 50 ml white spirit per month and continued for 6 months were regarded as current drinkers. Current drinkers were divided into three groups: 1-20 g/d alcohol, 21-40g/d alcohol and >40 g/d alcohol. Tobacco smoking was categorized into former, never and current drinking. Individuals who quit smoking more than one year were considered as former smokers, individuals who smoked more than 20 packets of cigarettes per year, or smoked more than one cigarette per day and continued for 6 months were regarded as current smokers. Consumption of vegetable and fruit were categorized into three groups: Seldom, ≤3 times/ week and ≥4 times/ week. 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, drinking, vegetable and fruit. 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 81 cases and 162 controls was 57.2 and 56.1 years, respectively. The ESCC cancer patients were found to have significantly higher levels of drinking than controls, with the ORs(95%CI) of 3.13(1.09-9.14) and 4.09(1.95-8.69) in moderate and heavy drinkers, respectively, while had significantly lower risk of ESCC when high consumption of fruit, with the ORs of 0.27(0.08-0.89), respectively. No significant lower ESCC risk was found in high consumption of vegetable (≤3 times/ week).

The genotype distributions of ADH1B and ALDH2 were in Hardy-Weinberg equilibrium in both cases and controls (ADH1B, P=0.24 and 0.65, respectively; ALDH2, p=0.03 and 0.15, respectively). When compared with the ADH1B*2/*2 genotype, the subjects carrying ADH1B*1/*2 and ADH1B*1/*1 increased the risk of ESCC, and the ORs(95%CI) were 1.47(0.84-2.58) and 2.36(1.14-5.79), respectively. For ALDH2 genotype, the ALDH2*1/*2 genotype was significantly associated with increased ESCC risk compared with ALDH2*1/*1 [OR(95%)=3.24(1.45-5.36)], and ALDH2*2/*2 showed no significantly decreased ESCC risk [OR(95%)=0.65(0.22-2.18)].

The risk of ESCC stratified by alcohol drinking status

Table 1. Characteristics of Patients and Controls

Characteristic	Cases n=81(%)	Controls n=162(%)	P	OR
Age				
<50	17(21)	34(21)	-	-
50-64	38(47)	76(47)		-
≥65	26(32)	52(32)		-
Mean age±SD	57.2±9.4	56.1±7.3	>0.05	-
Drinking status				
Former	4(5)	1(1)	<0.004	9.41(0.88-468)
Never	37(46)	87(54)		Reference
Light	24(30)	63(39)		0.90(0.46-1.71)
Moderate	12(15)	9(6)		3.13(1.09-9.14)
Heavy	4(5)	2(1)		4.70(0.63-53.4)
Smoking status				
Former	2(2)	3(1)	<0.001	1.79(0.14-16.0)
Never	53(65)	142(88)		Reference
Current	26(32)	17(10)		4.09(1.95-8.69)
Vegetable				
Seldom	2(2)	2(1)	0.633	Reference
≤3 times/ week	41(51)	76(47)		0.54(0.38-7.74)
≥4 times/week	38(47)	84(52)		0.45(0.03-6.50)
Fruit				
Seldom	11(14)	9(6)	0.04	Reference
≤3 times/ week	54(67)	105(65)		0.42(0.15-1.20)
≥4 times/week	16(20)	48(30)		0.27(0.08-0.89)

Table 2. ADH1B and ALDH2 Gene Polymorphisms and ESCC Risk

Gene polymorphisms	Cases n=81	Controls n=162	OR[1]
ADH2			
*2/*2	33(39)	78(48)	Reference
*1/*2	34(42)	67(42)	1.47(0.84-2.58)
*1/*1	15(19)	17(10)	2.36(1.14-5.79)
*1/*2+*1/*1	49(61)	84(52)	1.55(0.87-2.81)
ALDH2			
*1/*1	36(44)	85(52)	Reference
*1/*2	42(52)	60(37)	3.24(1.45-5.36)
*2/*2	3 (4)	18(11)	0.65(0.22-2.18)
*1/*2+*2/*2	45(56)	78(48)	1.85(0.87-2.34)
Allele frequencies			
ADH1B			
*2	100(123)	223(138)	Reference
*1	62(77)	101(62)	1.51(0.93-2.37)
ALDH2			
*1	114(141)	230(142)	Reference
*2	48(59)	96(59)	1.73(0.81-2.79)

[1]Adjusted for age, drinking, smoking, vegetable and fruit

in each ADH1B and ALDH2 genotype was showed in table 3. The risk of ESCC was moderately increased in heavy drinkers with ADH1B*1/*1 compared with never drinkers with ADH1B*2, and the adjusted OR (95% CI) was 4.70(0.34-311.74). We did not find significant increased trend of esophageal cancer risk in terms of alcohol categories in ADH1B*1/*1 (P for trend: 0.074). For ALDH2 genotype, ALDH2*1/*2 was associated with a no significantly heavy ESCC risk in heavy drinkers [OR(95%CI)= 8.55(0.48-412.4)], indicating a so called gene-environment interaction. Additionally, a significantly increased trend in cancer risk was observed throughout the categories of alcohol drinking in women carrying ALDH2*1/*2 (P for trend: 0.034 and 0.002, respectively).

Discussion

This study suggests that risk of ESCC was significantly related to ADH1B and ALDH2 genotypes in women, especially in female heavy drinkers. ADH1B*1/*2 increased the risk of ESCC development in female alcohol drinkers. ALDH2*1/*2 carriers showed a strong ESCC risk in heavy female drinkers.

Previous study reported esophageal cancer is associated with alcohol drinking (Castellsague et al., 1999; Gallus et al., 2001; Engel et al., 2003), so it is reasonable to assume that ADH1B and ALDH2 genotypes in female drinkers may affect the risk of esophageal cancer. A previous meta-analysis study indicated the inactive ADH1B and ALDH2 had 3.55 folds and 6.85 folds of esophageal cancer risk than active genotypes in female heavy drinkers(Yang et al., 2010). Also, a study conducted in Japan showed ORs of ALDH2*1/*2 heterozygotes for light, moderate and heavy drinking were the ORs for light, moderate, and heavy drinking were 4.12 (1.07–15.9), 9.87 (1.42–68.6), and 124.8 (9.48–1000<), respectively, in women and 8.11 (2.12–31.0), 75.6 (19.9–288.1), and 127.6 (32.7–497.3), respectively, in men(Yokoyama et al., 2002; Yokoyama et al., 2006). Studies conducted in China showed the ORs of esophageal cancer in male moderate to heavy male drinkers with ADH1B*1/*1 and ALDH2*1/*2 were at the range of 3.95 to 4.83 and 3.53 to 13.30, respectively(Ding et al., 2009; Yang et al., 2010). While, our study showed ORs were 2.71 to 3.70 and 1.93 to 7.05, which indicated the drinking women with inactive ADH1B and ALDH2 shared the relatively lower risk of

Table 3. Risk of ESCC Stratified by Alcohol Drinking Status in ADH1B and ALDH2 Genotypes

	ADH1B*2		OR	ADH1B*1/*1		OR
	Cases n=67	Controls n=145		Cases n=15	Controls n=17	
Former	2	1	-	2	0	-
Never	33	77	Reference	4	10	0.97(0.21-3.62)
Light	21	59	1.03(0.49-2.03)	3	4	1.76(0.31-11.7)
Moderate	9	7	1.69(1.24-6.58)	3	2	2.71(0.41-44.2)
Heavy	2	1	2.72(0.55-79.6)	2	1	3.70(0.34-281.7)
	ALDH2*1/*1			ALDH2*1/*2		
	Cases n=42	Controls n=60		Cases n=85	Controls n=36	
Former	1	1	-	3	1	-
Never	23	57	Reference	18	29	1.59(0.87-3.71)
Light	7	21	0.92(0.32-2.75)	16	26	2.17(1.29-4.52)
Moderate	4	4	2.27(0.44-19.4)	2	3	1.93(0.29-16.6)
Heavy	1	2	1.03(0.07-27.5)	3	1	7.05(0.48-331.4)

esophageal cancer than men.

The explanation of different risk of ESCC between men and women could be explained by the following reasons. Firstly, the limited sample size of female cancer patients, especially low frequencies of drinkers among cases and controls may decrease the statistical power. A larger sample study or a large number of controls study are needed to increase the study power to allow us a more detailed analysis to find the ESCC risk in women. Secondly, women are more likely to have a health life habits than men, with regard to high intake of fruit and vegetable, low frequency of smoking. These health habits would diminish the effects of alcohol drinking and ethanol and acetaldehyde exposure in women. Additionally, the hormonal factors may also play an important role in the cancer susceptibility. Recent studies from England and Switzerland found hormone replacement therapy and long time breastfeeding were inversely related to the risk of esophageal cancer(Cheng et al., 2000; Lagergren and Jansson, 2005). Our study did not concern the effect of hormone on ESCC in women. Further study is need to measure the hormone status to clarify whether there are any gender differences when exposure to similar ethanol and acetaldehyde.

Our study has several major strengthens. Firstly, we collected the information of the major risk of ESCC, and these factors were adjusted throughout the analysis. Secondly, all the interviewers used the same query mode for each question to avoid measurement bias. Thirdly, In many case-control studies, controls were usually taken from inpatients or outpatients who would have more chance of same exposure of cases than generally population. However, all the controls in our study were selected from those who came to hospitals for routine health examination, which would be better representative of the general population than the hospitalized controls.

In conclusion, our study showed an increased risk of ESCC in female with inactive ADH1B and ALDH2 genotypes. Female drinkers had a similar strong risk of ESCC as that seemed in males, but no significantly increased cancer risk was found in our study due to the limited sample size. We could conclude that women with inactive ADH1B and ALDH2 should have less drinking, higher intake of vegetable and fruit to prevent the development of esophageal cancer.

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