A variant allele of *ADH1B* and *ALDH2* is associated with the risk of esophageal cancer

HAIYONG GU[1*], DINGXU GONG[2*], GUOWEN DING[1], WENBO ZHANG[3], CHAO LIU[1], PENGCHENG JIANG[3], SUOCHENG CHEN[1] and YIJANG CHEN[4]

[1]Department of Cardiothoracic Surgery, Affiliated People's Hospital of Jiangsu University, Zhejiang 212000; [2]Department of Surgery, State Key Laboratory of Translational Cardiovascular Medicine, Fuwai Hospital and Cardiovascular Institute, Chinese Academy of Medical Science, Peking Union Medical College, Beijing 100037; [3]Department of General Surgery, Affiliated People's Hospital of Jiangsu University, Zhejiang 212000; [4]Department of Thoracic and Cardiac Surgery, First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, P.R. China

Received November 17, 2011; Accepted April 11, 2012

DOI: 10.3892/etm.2012.547

Abstract. Alcoholic beverages are causally related to esophageal cancer. The genetic polymorphisms of the alcohol-metabolizing enzymes *ADH1B* rs1229984 and *ALDH2* rs671 may modulate individual differences in alcohol-oxidizing capability. A case-control study was conducted to evaluate the genetic effects of these two functional single nucleotide polymorphisms (SNPs) on the development of esophageal cancer. A total of 380 esophageal squamous cell carcinoma cases and 380 controls were recruited. Genotypes were determined by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Variant alleles of the functional polymorphism *ADH1B* rs1229984 SNP were associated with an increased risk of esophageal cancer [adjusted odds ratio (OR)=2.39, 95% confidence interval (CI)=1.42-4.03 for *ADH1B* rs1229984 GG vs. AA]. There was a borderline-significantly decreased risk between the *ALDH2* rs671 genotype and esophageal cancer (adjusted OR=0.47, 95% CI=0.22-1.00 for *ALDH2* rs671 AA vs. GG). Stratified analyses indicated that both of these effects were more evident among male, younger subjects and smokers. In conclusion, the functional polymorphisms *ADH1B* rs1229984 and *ALDH2* rs671 may contribute to susceptibility to esophageal cancer, particularly among male, younger subjects and smokers.

Correspondence to: Professor Suocheng Chen, Department of Cardiothoracic Surgery, Affiliated People's Hospital of Jiangsu University, Zhejiang 212000, P.R. China
E-mail: chensuocheng@sina.com

Dr Yijang Chen, Department of Thoracic and Cardiac Surgery, The First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, P.R. China
E-mail: yjchen@njmu.edu.cn

[*]Contributed equally

Abbreviations: ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; SNP, single nucleotide polymorphism

Key words: ADH1B, *ALDH2*, polymorphisms, esophageal cancer, molecular epidemiology

Introduction

Esophageal cancer is an extremely aggressive cancer, of which China has high-incidence regions (1). Esophageal squamous cell carcinoma (ESCC) is a subtype of esophageal cancer which accounts for >90% of cases (2). Esophageal cancer is known to be associated with environmental carcinogens. Epidemiological studies indicate that use of tobacco and consumption of alcohol are major risk factors for esophageal cancer. However, only a subset of individuals exposed to tobacco and alcohol develop esophageal cancer, suggesting a role of host susceptibility factors in cancer development. The genetic basis of esophageal cancer is complex and appears to involve multiple genes. Some studies have suggested that genetic polymorphisms might explain individual differences in susceptibility to esophageal cancer (3).

Alcohol intake may be causally related to cancer of the oral cavity, pharynx, larynx and esophagus. Ethanol is oxidized to acetaldehyde and then to acetate by alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH); both of which have genetic polymorphisms. The genetic polymorphisms of alcohol-metabolizing enzymes modulate individual differences in alcohol-oxidizing capability and drinking behavior (4).

In humans, the major enzymes involved in the alcohol-metabolizing pathways are alcohol dehydrogenase 1B (ADH1B) and aldehyde dehydrogenase 2 (ALDH2). Alcohol is first oxidized by ADH to acetaldehyde, which is oxidized to acetate by ALDH. These enzymes are mainly expressed in the liver, but are also present in the gastrointestinal tract (5).

The gene encoding the ADH1B enzyme is located on chromosome 4q22, and the *ADH1B* gene (encoding for subunit β) is the locus responsible for the majority of the ADH activity on ethanol in the liver (6). It has been predicted that individuals expressing variants of *ADH1B* in particular could have different rates of alcohol elimination (7). ADH1B is a low K_m (Michaelis constant)-class enzyme and exhibits high activity in catalyzing ethanol to acetaldehyde (6). The most frequently reported locus is *ADH1B* Arg47His (rs1229984). In *ADH1B* His/His individuals, which are associated with flushing or other reactions to alcohol, the activity of ADH1B has been demonstrated to be decreased by 40-fold (8).

The sequence variant (rs671) on chromosome 12q24.2 was found to be associated with inactive ALDH2. A mutant allele, *ALDH2* AA, has a single point mutation (G→A transition in exon 12) at position 1510 of the active *ALDH2* GA gene. This results in the substitution of glutamic acid 504 to lysine, and therefore produces inactive ALDH2 (4,9,10).

A recent genome-wide association study identified the variation of *ADH1B* rs1229984 and *ALDH2* rs671 polymorphisms as risk factors for esophageal cancer (11). Another genome-wide association study reported that variations of *ADH1B* rs1229984 and *ALDH2* rs671 coupled with alcohol drinking and smoking synergistically enhanced the risk of esophageal cancer (12).

Due to the biological and pathological significance of ADH1B and ALDH2, functional genetic variations in the *ADH1B* and *ALDH2* genes may contribute to the development of esophageal cancer. We evaluated the association between *ADH1B* and *ALDH2* genotypes and susceptibility to esophageal cancer in a hospital-based case-control study. Genotyping analyses were conducted for the two SNPs with 380 ESCC cases and 380 controls in a Chinese population.

Patients and methods

Ethical approval of the study protocol. This hospital-based case-control study was approved by the Review Board of Jiangsu University (Zhenjiang, China). All subjects provided written informed consent prior to inclusion in the study.

Study subjects. A total of 380 subjects with esophageal cancer were consecutively recruited from the Affiliated People's Hospital of Jiangsu University and Affiliated Hospital of Jiangsu University (Jiangsu, China) between October 2008 and November 2009. All cases of esophageal cancer were diagnosed as ESCC by pathological means. The exclusion criteria were patients who previously had cancer, any metastasized cancer, radiotherapy or chemotherapy. The controls were patients without cancer who were frequency-matched to the cases with regard to age (±5 years) and gender, and were recruited from the two abovementioned hospitals during the same period. The majority of the control subjects had trauma or infectious diseases.

Each subject was personally questioned by trained interviewers using a pre-tested questionnaire to obtain information on demographic data (e.g., age, gender) and related risk factors (including tobacco smoking and alcohol consumption). Following the interview, 2 ml samples of venous blood were collected from each subject. Individuals who smoked one cigarette per day for >1 year were defined as 'smokers'. Subjects who consumed ≥3 alcoholic drinks a week for >6 months were considered to be 'alcohol drinkers'.

Isolation of DNA and genotyping by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-ToF-MS). Blood samples were collected from patients using Vacutainers and transferred to tubes lined with ethylenediaminetetraacetic acid (EDTA). Genomic DNA was isolated from whole blood using the QIAamp DNA Blood Mini kit (Qiagen, Berlin, Germany). Genotyping was conducted by MALDI-ToF-MS as previously described (13). SNP genotyping was performed using the MassArray system (Sequenom, San Diego, CA, USA) by MALDI-ToF-MS according to the manufacturer's instructions. Completed genotyping reactions were spotted onto a 384-well spectroCHIP system (Sequenom) using a MassArray Nanodispenser (Sequenom) and determined by MALDI-ToF-MS. Genotype calling was performed in real time with MassArray RT software version 3.1 (Sequenom), and analyzed using MassArray Typer software version 4.0 (Sequenom) (Figs. 1 and 2). For quality control, repeated analyses were conducted for 10% of randomly selected samples.

Statistical analyses. Differences in the distributions of demographic characteristics, selected variables and genotypes of *ADH1B* and *ALDH2* variants between cases and controls were evaluated using the χ^2 test. Associations between *ADH1B* and *ALDH2* genotypes and the risk of esophageal cancer were estimated by computing the odds ratios (ORs) and their 95% confidence intervals (CIs) using logistic regression analyses for crude ORs and adjusted ORs when adjusting for age, gender, tobacco use and drinking status. The Hardy-Weinberg equilibrium was tested by a goodness-of-fit χ^2 test to compare the observed genotype frequencies to the expected among the control subjects. All statistical analyses were conducted using SAS 9.1.3 software (SAS Institute, Cary, NC, USA).

Results

Characteristics of the study population. Among the 380 ESCC cases and the 380 controls with DNA samples, genotyping was successful in 379 (99.7%) cancer cases and 378 (99.5%) controls for *ADH1B* rs1229984. For *ALDH2* rs671, genotyping was successful in 380 (100.0%) cancer cases and 378 (99.5%) controls. Characteristics of cases and controls are summarized in Table I. Cases and controls appeared to be adequately matched with respect to age and gender as suggested by the χ^2 tests (p=0.056 and 0.346, respectively). No significant difference was observed with regard to drinking status between cases and controls (p=0.183). However, the prevalence of smoking was higher in the esophageal cancer patients than in the control subjects (p=0.014) (Table I).

Associations between ADH1B rs1229984 and ALDH2 rs671 polymorphisms and the risk of esophageal cancer. The genotype distributions of *ADH1B* and *ALDH2* in the cases and controls are shown in Table II. The observed genotype frequencies for these two polymorphisms in the controls were

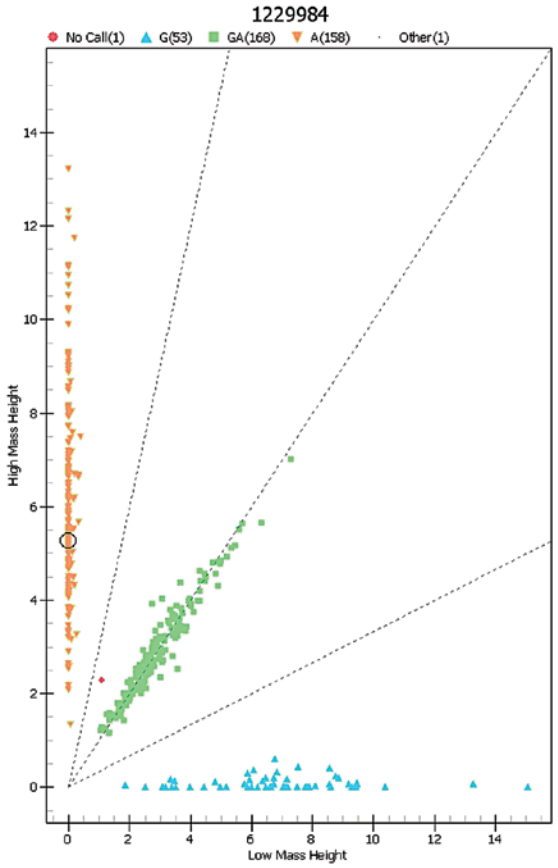

Figure 1. Genotyping of ADH1B rs1229984 A/G by MALDI-ToF-MS. ADH1B, alcohol dehydrogenase 1B; MALDI-ToF-MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

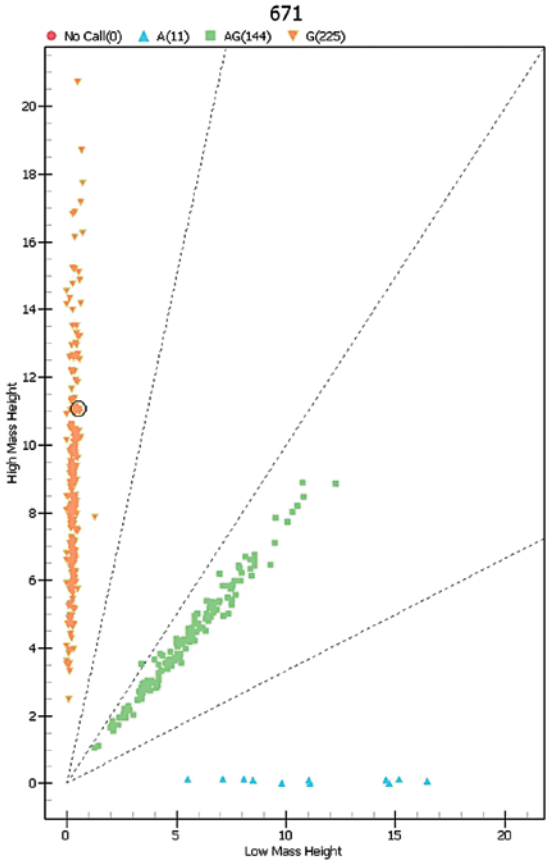

Figure 2. Genotyping of ALDH2 rs671 G/A by MALDI-ToF-MS. ALDH2, aldehyde dehydrogenase 2; MALDI-ToF-MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

Table I. Distribution of selected demographic variables and risk factors in esophageal cancer cases and control subjects.

	Cases (n=380)		Controls (n=380)		
Variable	n	%	n	%	p-value[a]
Age (years)					0.056
<60	142	37.4	117	30.8	
≥60	238	62.6	263	69.2	
Gender					0.346
Male	269	70.8	257	67.6	
Female	111	29.2	123	32.4	
Tobacco use					0.014
Never smoked	220	57.9	253	66.6	
Have smoked	160	42.1	127	33.4	
Alcohol use					0.183
Never consumed	253	66.6	270	71.1	
Have consumed	127	33.4	110	28.9	

[a]Two-sided χ^2 test.

all within Hardy-Weinberg equilibrium (p=0.102 and 0.925 for *ADH1B* and *ALDH2*, respectively). In the single-locus analyses, the genotype frequencies of *ADH1B* rs1229984 were 41.7% (AA), 44.3% (AG) and 14.0% (GG) in the patients, and 48.1% (AA), 45.0% (AG) and 6.9% (GG) in the control subjects. The difference was revealed to be significant (p=0.004). Logistic regression analyses revealed that subjects carrying the GG variant homozygote had a significant 2.39-fold (adjusted OR=2.39; 95% CI=1.42-4.03) increased risk of esophageal cancer. However, the genotype frequencies of *ALDH2* rs671 were not significantly different between the cases and the controls (p=0.141). Logistic regression analyses revealed that the *ALDH2* rs671 AA variant genotype, but not the *ALDH2* rs671 GA heterozygote, was associated with a borderline-significantly decreased risk of esophageal cancer (adjusted OR=0.47, 95% CI=0.22-1.00 for rs671 AA and adjusted OR=0.99, 95% CI=0.73-1.34 for rs671 GA, respectively), compared with the rs671 GG wild-type homozygote.

In the recessive model, the *ADH1B* rs1229984 GG variant homozygote was associated with a 2.20-fold significantly increased risk of esophageal cancer compared with rs1229984 AA/AG genotypes (adjusted OR=2.20, 95% CI=1.34-3.60). However, for the *ALDH2* rs671 G/A polymorphism, the rs671 AA genotype was associated with a significantly decreased risk of esophageal cancer (adjusted OR=0.47, 95% CI=0.22-1.00), compared with rs671 GG/GA genotypes.

Stratification analyses of ADH1B rs1229984 and ALDH2 rs671 polymorphisms and risk of esophageal cancer. Stratification

Table II. Logistic regression analyses of associations between *ADH1B* and *ALDH2* polymorphisms and risk of esophageal cancer.

	Cases[a] (n=380)		Controls (n=380)					
Genotype	n	%	n	%	Crude OR (95% CI)	p-value	Adjusted OR[b] (95% CI)	p-value
ADH1B rs1229984								
AA	158	41.7	182	48.1	1.00		1.00	
AG	168	44.3	170	45.0	1.14 (0.84-1.54)	0.400	1.14 (0.84-1.54)	0.399
GG	53	14.0	26	6.9	**2.35 (1.40-3.93)**	**0.001**	**2.39 (1.42-4.03)**	**0.001**
AA+AG	326	86.0	352	93.1	1.00		1.00	
GG	53	14.0	26	6.9	**2.20 (1.34-3.60)**	**0.002**	**2.23 (1.36-3.68)**	**0.002**
G allele		36.1		29.4				
ALDH2 rs671								
GG	225	59.2	219	57.9	1.00		1.00	
GA	144	37.9	137	36.2	1.02 (0.76-1.34)	0.881	0.99 (0.73-1.34)	0.956
AA	11	2.9	22	5.8	0.49 (0.23-1.03)	0.059	0.47 (0.22-1.00)	0.050
GG+GA	369	97.1	356	94.2	1.00		1.00	
AA	11	2.9	22	5.8	0.48 (0.23-1.01)	0.053	**0.47 (0.22-1.00)**	0.048
A allele		21.8		23.9				

[a]Genotyping was successful in 379 (99.7%) cancer cases, and 378 (99.5%) controls for *ADH1B* rs1229984. For *ALDH2* rs671, genotyping was successful in 380 (100.0%) cancer cases and 378 (99.5%) controls. [b]Adjusted for age, gender, smoking and drinking status. CI, confidence interval; OR, odds ratio; *ADH1B*, alcohol dehydrogenase 1B; ALDH2, aldehyde dehydrogenase 2. Bold type indicates statistical significance (p<0.05).

Table III. Stratified analyses between *ADH1B* and *ALDH2* polymorphisms and risk of esophageal cancer by gender, age, smoking status and alcohol consumption.

	ADH1B rs1229984 (case/control)		OR[a] (95% CI)		*ALDH2* rs671 (case/control)		OR[a] (95% CI)	
Variable	AA+AG	GG	AA+AG	GG	GG+GA	AA	GG+GA	AA
Gender								
Male	226/238	43/17	1.00	**2.72 (1.50-4.95)**	266/237	3/18	1.00	**0.13 (0.04-0.47)**
Female	100/114	10/9	1.00	1.26 (0.49-3.27)	103/119	8/4	1.00	2.40 (0.70-8.26)
Age (years)								
<60	118/109	24/8	1.00	**2.41 (1.29-4.48)**	139/107	3/10	1.00	**0.24 (0.06-0.92)**
≥60	208/243	29/18	1.00	1.87 (0.79-4.40)	230/249	8/12	1.00	0.68 (0.27-1.71)
Smoking status								
Never smoked	195/235	24/17	1.00	1.84 (0.95-3.56)	210/241	10/12	1.00	1.05 (0.44-2.51)
Have smoked	131/117	29/9	1.00	**3.24 (1.44-7.28)**	159/115	1/10	1.00	**0.05 (0.01-0.38)**
Alcohol consumption								
Never consumed	227/252	25/17	1.00	1.90 (0.99-3.65)	242/253	11/16	1.00	0.67 (0.30-1.52)
Have consumed	99/100	28/9	1.00	**3.05 (1.35-6.90)**	127/103	0/6	1.00	Not available

[a]Adjusted for age, gender, smoking status and alcohol consumption in a logistic regression model. CI, confidence interval; OR, odds ratio. ADH1B, alcohol dehydrogenase 1B; ALDH2, aldehyde dehydrogenase 2. Bold type indicates statistical significance.

analyses were conducted to evaluate the effects of ADH1B and ALDH2 genotypes on the risk of esophageal cancer according to age, gender, smoking status and alcohol-consumption status (Table III). A significantly increased risk of esophageal cancer associated with the *ADH1B* rs1229984 GG genotype was evident among males (adjusted OR=2.72, 95% CI=1.50-4.95)

aged <60 years (adjusted OR=2.41, 95% CI=1.29-4.48), smokers (adjusted OR=3.24, 95% CI=1.44-7.28), and consumers of alcohol (adjusted OR=3.05, 95% CI=1.35-6.90), compared with the rs1229984 AA/AG genotype. For the *ALDH2* rs671 variant, the risk effects of rs671 AA vs. rs671 GG/GA were significant in males (adjusted OR=0.13, 95% CI=0.04-0.47), younger subjects (<60 years) (adjusted OR=0.24, 95% CI=0.06-0.92), and smokers (adjusted OR=0.05, 95% CI=0.01-0.38).

Discussion

We investigated the associations of *ADH1B* and *ALDH2* SNPs with risk of esophageal cancer in a high-risk Chinese population. Multivariable logistic analyses revealed that the *ADH1B* rs1229984 GG genotype was associated with an increased risk, and that the *ALDH2* rs671 AA genotype was associated with a significantly decreased risk of esophageal cancer, and that this effect was more evident among males, younger subjects and smokers. We found a significant gene-environment interaction between exposure to smoking and *ADH1B* and *ALDH2* SNPs for the risk of esophageal cancer. Our results suggest a potential role of *ADH1B* and *ALDH2* SNPs on the etiology of esophageal cancer.

Two recent genome-wide association studies identified the variation of *ADH1B* rs1229984 and *ALDH2* rs671 polymorphism as risk factors for esophageal cancer in a Japanese population (11,12). However, in another three genome-wide association studies in larger Chinese populations, the results presented negative or protective efforts of these polymorphisms for esophageal cancer risk (2,3,14). The reason for these inconsistent findings for *ADH1B* rs1229984 and *ALDH2* rs671 polymorphisms is unknown. However, variation in enzyme activity with ethnicity and gender could contribute to differences in influences on neoplasms. The genome instability induced by ethanol- and acetaldehyde-mediated pathways could explain *ADH1B* and *ALDH2* polymorphic effects on alcohol-induced carcinogenesis (15). In the present study, we also found a significant gene-environment interaction between *ADH1B* rs1229984 and *ALDH2* rs671 polymorphisms and smoking habit, suggesting susceptibility to esophageal cancer.

Alcoholic beverages may have carcinogenic effects on humans and are causally related to cancer of the oral cavity, pharynx, larynx and esophagus (10). The genetic polymorphisms of alcohol-metabolizing enzymes modulate individual differences in alcohol-oxidizing capability and drinking behavior (4). Individuals with the *ALDH2* GA/AA genotype should have only 6.25% of normal *ALDH2* GG protein, and molecules containing one or more *ALDH2* A subunits are considered to be inactive (9). How enzyme polymorphisms influence individual cancer susceptibility is a new area of research.

Distribution of the *ALDH2* rs671 allele varies with ethnicity. The *ALDH2* rs671 allele is prevalent in subjects in East Asia, but has not been found in Caucasians or Africans (16). However, this variant is common in Asians, with 30-40% of the population being heterozygous (*ALDH2* GA) and 2.5-5% being homozygous for the null variant (*ALDH2* AA) (17). The allele frequency of the *ALDH2* rs671 polymorphism in our control population (36.2% for GA and 5.8% for AA) was similar to results observed in Asians. The observed genotype frequency for the *ALDH2* rs671 polymorphism in the controls was within Hardy-Weinberg equilibrium (p=0.925), indicating a good representation for our control population.

A recent study found that there was no significant impact of *ADH1B* rs1229984 and *ALDH2* rs671 polymorphisms on the risk of breast cancer. Neither was there a significant gene-environment interaction between alcohol consumption and polymorphisms in *ADH1B* rs1229984 and *ALDH2* rs671 (18). In a recent study in a Chinese Han population, Gao *et al* found that rs671 A/G and A/A genotypes were protective against the risk of colorectal cancer (19). However another study by Sangrajrang *et al* did not find any affect of the *ALDH2* rs671 polymorphism on the risk of breast cancer (20). Two studies in Asian populations found a significantly higher risk of cancer of the upper aerodigestive tract (UADT), oral cavity or oropharynx and hypopharynx in moderate or heavy drinkers of alcohol carrying the *ADH1B* $^{*}1/^{*}1$ (GG) genotype (21,22).

Hashibe *et al* identified the variation of *ADH1B* rs1229984 as a risk factor for esophageal cancer in European and Latin American populations (23). Their results were consistent with the results of the present study and another investigation conducted by Tanaka *et al* in a Japanese population (11). A recent meta-analysis conducted by Guo *et al* revealed that the *ADH1B* 47Arg (G) allele was a common genetic variant that increased the risk of cancers of the UADT, while also modulating the susceptibility to UADT cancers coupled with alcohol drinking (24). In another two meta-analyses investigating the *ADH1B* rs1229984 polymorphism and esophageal cancer, genetic variations of *ADH1B* His47Arg (A/G) were also found to be susceptible loci for esophageal cancer (25,26). The conclusions were in accordance with our results. For the *ADH1B* rs1229984 polymorphism, the observed genotype frequencies in the controls were also within Hardy-Weinberg equilibrium (p=0.102).

The present study has several limitations. Firstly, it was a hospital-based case-control study, therefore selection bias may be unavoidable and the subjects may not be representative of the general population. Secondly, the polymorphisms investigated were based on functional considerations, so they may not give a comprehensive view about the genetic variability in *ADH1B* and *ALDH2*. Thirdly, the present study involved a relatively small number of subjects in the subgroup analyses. This may have resulted in reduction in the magnitude of the statistical power, with an increase in the potential for random error. Therefore, larger well-designed studies are required to confirm our findings. Finally, we did not obtain detailed information on cancer metastasis and survival, which restricted further analyses on the role of *ADH1B* and *ALDH2* polymorphisms in the progression and prognosis of esophageal cancer.

In conclusion, the present study provided marked evidence that functional polymorphism of *ADH1B* rs1229984 and *ALDH2* rs671 may contribute to the risk of esophageal cancer. However, our results were obtained with a limited sample size and therefore only preliminary conclusions can be drawn. Validations of these findings with further larger studies and more diversely ethnic populations are required.

Acknowledgements

This study was supported in part by National Natural Science Foundation of China (81101889) and Social Development Foundation of Zhenjiang (SH2010017).

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