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Analysis of *ADH1B* Arg47His, *ALDH2* Glu487Lys, and *CYP4502E1* polymorphisms in gastric cancer risk and interaction with environmental factors

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ABSTRACT. Gastric cancer is the fourth commonly diagnosed cancer and the second most frequent cause of cancer death worldwide. Genetic variations in *ADH1B* and *ALDH2* may alter the function and activity of the corresponding enzymes, leading to differences in acetaldehyde exposure between drinkers. Cytochrome P4502E1 (*CYP4502E1*) is a phase I enzyme that plays an important role in metabolizing nitrosamine compounds and the bioactivation of procarcinogens. During the period of July 2013 to July 2015, 246 patients and 274 controls were enrolled from the First Affiliated Hospital of Jinan University. In the codominant model, the AA genotype of *ALDH2* Glu487Lys significantly elevated the risk of gastric cancer in comparison with the GG genotype of *ALDH2* Glu487Lys. In the recessive model, the AA genotype of *ALDH2* Glu487Lys significantly increased the risk of gastric cancer compared to the GG+GA genotype (OR = 2.34 95%CI = 1.02-5.70). We found in the codominant model that individuals harboring the C2/

C2 genotype of *CYP4502E1* had a higher risk of developing gastric cancer than those with the C1/C1 genotype. In addition, in the recessive model, we found that the C2/C2 genotype correlated with an elevated risk of gastric cancer in comparison with the C1/C1+C1/C2 genotype (OR = 4.90, 95%CI = 2.04-13.51). However, no significant relationship was measured between *ADH1B* Arg47His and gastric cancer risk. In summary, the results of our study indicate that *ALDH2* Glu487Lys and *CYP4502E1* polymorphisms could be risk factors for the development of gastric cancer in the Chinese population.

Key words: ADH1B Arg47His; ALDH2 Glu487Lys; CYP4502E1; Polymorphism; Gastric cancer

INTRODUCTION

Gastric cancer is the fourth most commonly diagnosed cancer and the second most frequent cause of cancer death worldwide (International Agency for Research on Cancer, 2012). Many environmental and lifestyle factors are involved in the development of gastric cancer, including *Helicobacter pylori*, heavy smoking and drinking (Khayatzadeh et al., 2015; den Hoed and Kuipers, 2016; Lee et al., 2016). The incidence of gastric cancer varies greatly across different populations even when they are exposed to similar environmental risk factors, implying that hereditary factors influence the pathogenesis of this cancer. An increasing number of genomic studies have reported that many genetic factors contribute to the development of gastric cancer (Shi et al., 2015; Lin et al., 2016; Xia et al., 2016).

After consumption of an alcoholic beverage, ADH1B first catalyzes ethanol into acetaldehyde, a highly reactive and toxic substance. Acetaldehyde is oxidized to acetic acid by ALDH2. Acetic acid participates in the Krebs cycle, is metabolized into CO_2 and H_2O, and is excreted from the body (Dakeishi et al., 2008; Kang et al., 2009; Lee et al., 2015). Genetic variations in *ADH1B* and *ALDH2* may alter the function and activity of the corresponding enzymes, leading to differences in acetaldehyde exposure between drinkers (Dakeishi et al., 2008; Lai et al., 2013; Lee et al., 2015). Few studies have explored the correlation between *ADH1B* and *ALDH2* and the risk of gastric cancers in Japanese and Chinese populations (Cao et al., 2010; Duell et al., 2012; Wang et al., 2014; Hidaka et al., 2015). Moreover, cytochrome P4502E1 (*CYP4502E1*) is a phase I metabolizing enzyme that plays an important role in metabolizing nitrosamine compounds and converting procarcinogens to activate carcinogens. Polymorphisms in *CYP4502E1* could cause individualized susceptibility to cancer (Qin et al., 2008). However, till date, no study has reported the correlation between *CYP4502E1* polymorphism and the risk of gastric cancer; therefore, we carried out a case-control study to explore the role of *ADH1B* Arg47His, *ALDH2* Glu487Lys, and *CYP4502E1* polymorphisms in the development of gastric cancer in a Chinese population.

MATERIAL AND METHODS

Selection of subjects

Between July 2013 to July 2015, 246 patients were enrolled from the First Affiliated

Hospital of Jinan University. All patients with gastric cancer underwent upper gastrointestinal endoscopy and pathological examination by pathologists. Patients undergoing chemotherapy before enrollment were excluded from this study. Gastric cancer patients with metastasis or recurrent tumors, malnutrition, and end-stage liver or kidney diseases were excluded from the study.

The 274 control subjects were healthy individuals that underwent upper gastrointestinal endoscopy, and were confirmed to be free of any malignant tumors and digestive, end-stage liver or kidney, and metabolism-related diseases. The control subjects were enrolled from the patient clinic of the department of gastroenterology at the First Affiliated Hospital of Jinan University.

The demographic information and clinical variables of all investigated participants were collected through medical records or a questionnaire. They involved gender, age, family history of cancer, habit of tobacco smoking and alcohol drinking and clinical stage of gastric cancer. All subjects signed an informed consent form before enrollment, and the performance of this study was approved by the Ethics Committee of the First Affiliated Hospital of Jinan University.

Genotyping

Five milliliters of peripheral blood was obtained from each participant. The blood samples were kept in a refrigerator at 4°C until utilization. The DNA was extracted by the Blood DNA kit produced by Tiangen Biotech Co., Ltd. (Beijing, China). The genotyping of *ADH1B* Arg47His, *ALDH2* Glu487Lys, and *CYP4502E1* were performed through polymerase chain reaction (PCR)-restriction fragment length polymorphism. The primers were provided by Sangon Biotech Co., Ltd. (Shanghai, China). The forward and reverse primer sequences for *ADH1B* Arg47His were 5'-AATCTTTTGTGAATCTGAACAG-3' and 5'-GAAGGGGGGTCACCAGGTTG-3', respectively. The forward and reverse primers for *ALDH2* Glu487Lys were 5'-GTCAACTGCTATGATGTGTTTGG-3' and 5'-CCACCAGCAGACCCTCAAG-3', respectively. The forward and reverse primer sequences for *CYP4502E1* were 5'-CCAGTCGAGTCTACATTGTCA-3' and 5'-TTCATTCTGTCTTCTAACTGG-3', respectively. The PCR amplification involved an initial denaturation at 97°C for 5 min, 35 cycles of denaturation at 94°C for 60 s, annealing at 57°C for 60 s and extension at 72°C for 60 s, and a final cycle of elongation at 72°C for 10 min.

PCR products of *ADH1B* Arg47His, *ALDH2* Glu487Lys, and *CYP4502E1* were digested using *Mae*III, *Eco*RI, and *Pst*I restriction enzymes, respectively. The amplification products were detected by Tiangen DNA markers (pUC18 DNA/*Msp*I).

Statistical analysis

Comparisons of the lifestyle characteristics and genotype frequencies of the two groups were performed by the chi-square test. Whether the genotype frequencies conformed to the Hardy-Weinberg equilibrium (HWE) was analyzed by the chi-square test. The correlation between *ADH1B* Arg47His, *ALDH2* Glu487Lys, and *CYP4502E1* polymorphisms and the risk of gastric cancer was analyzed by multiple regression analysis, and the results are reported by the odds ratio (OR) and 95% confidence intervals (CIs). Spearman's correlation analysis was employed to evaluate interactions between the two polymorphisms of interest and environmental factors. The codominant, dominant, and recessive models were used to assess

the association. All statistical analyses were two-sided tests, and a P value less than 0.05 was considered statistically significant.

RESULTS

Through comparison of the demographic and lifestyle variables between the two study groups, we observed that patients with gastric cancer had a habit of heavy smoking (χ^2 = 10.49, P = 0.01) and heavy drinking (χ^2 = 37.32, P < 0.001), a family history of cancer (χ^2 = 6.18, P = 0.01), and a habit of heavy intake of pickled food (χ^2 = 6.77, P = 0.03) (Table 1). However, the patients with gastric cancer and controls were comparable with respect to age (χ^2 = 0.53, P = 0.47) and gender (χ^2 = 0.44, P = 0.51). Of the 246 patients with gastric cancer, 105 (42.68%) cases were reported to be at the I-II stage and 141 (57.32%) cases at the III-IV stage.

Table 1. Demographic, lifestyle and clinical variables of gastric cancer patients and controls.

Variables	Patients (N = 246)	%	Controls (N = 274)	%	χ^2 value	P value
Age, years						
<60	100	40.65	120	43.80		
≥60	146	59.35	154	56.20	0.53	0.47
Sex						
Male	171	69.51	183	66.79		
Female	75	30.49	91	33.21	0.44	0.51
Smoking habit						
Never or few	114	46.34	153	55.84		
Moderate	63	25.61	76	27.74		
Heavy	69	28.05	45	16.42	10.49	0.01
Drinking habit						
Never or few	108	43.90	171	62.41		
Moderate	55	22.36	71	25.91		
Heavy	83	33.74	32	11.68	37.32	<0.001
Family history of cancer						
No	214	86.99	256	93.43		
Yes	32	13.01	18	6.57	6.18	0.01
Pickled food intake						
Never or few	154	62.60	188	68.61		
Moderate	52	21.14	62	22.63		
Heavy	40	16.26	24	8.76	6.77	0.03
Clinical stage						
I-II	105	42.68				
III-IV	141	57.32				

The genotype frequencies of *ADH1B* Arg47His, *ALDH2* Glu487Lys and *CYP4502E1* are shown in Table 2. Using the chi-square test, we observed that the C1/C1, C1/C2 and C2/C2 genotype distributions of *CYP4502E1* are significantly different between the two investigated groups (χ^2 = 16.50, P < 0.001). However, no significant differences are reported to be found between the two investigated groups in terms of *ADH1B* Arg47His (χ^2 = 1.13, P = 0.57) and *ALDH2* Glu487Lys (χ^2 = 4.02, P = 0.13) genotype frequencies. In addition, we observed that the genotype distributions of *ADH1B* Arg47His, *ALDH2* Glu487Lys and *CYP4502E1* are in line with HWE in both patient and control groups.

We used multiple logistic regression analysis to analyze the association between *ADH1B* Arg47His, *ALDH2* Glu487Lys and *CYP4502E1* polymorphisms and the risk of gastric cancer in three genetic models (Table 3).

Table 2. Genotype frequencies of *ADH1B* Arg47His, *ALDH2* Glu487Lys and *CYP4502E1* between the two groups.

Gene	Patients (N = 246)	%	Controls (N = 274)	%	χ^2 value	P value	χ^2 value for HWE	P value	χ^2 value for HWE	P value
							In patients		In controls	
ADH1B Arg47His										
GG	83	33.74	104	37.96						
GA	117	47.56	125	45.62						
AA	46	18.70	45	16.42	1.13	0.57	0.18	0.67	0.51	0.47
ALDH2 Glu487Lys										
GG	133	54.07	163	59.49						
GA	95	38.62	101	36.86						
AA	18	7.32	10	3.65	4.02	0.13	0.03	0.86	1.39	0.24
CYP4502E1										
C1/C1	121	49.19	156	56.93						
C1/C2	97	39.43	111	40.51						
C2/C2	28	11.38	7	2.55	16.50	<0.001	1.57	0.21	1.95	0.18

Table 3. Correlation between *ADH1B* Arg47His, *ALDH2* Glu487Lys and *CYP4502E1* polymorphisms and risk of gastric cancer.

Gene	Patients (N = 246)	%	Controls (N = 274)	%	Adjusted OR	P value
ADH1B Arg47His						
Codominant						
GG	83	33.74	104	37.96	1.0 (Reference)	-
GA	117	47.56	125	45.62	1.17 (0.79-1.75)	0.41
AA	46	18.70	45	16.42	1.28 (0.75-2.18)	0.33
Dominant						
GG	83	33.70	104	37.80	1.0 (Reference)	-
GA+AA	163	66.26	170	62.04	1.20 (0.83-1.75)	0.32
Recessive						
GG+GA	200	81.30	229	83.58	1.0 (Reference)	-
AA	46	18.70	45	16.42	1.17 (0.73-1.89)	0.50
ALDH2 Glu487Lys						
Codominant model						
GG	131	53.25	163	59.49	1.0 (Reference)	-
GA	95	38.62	101	36.86	1.17 (0.780-1.71)	0.44
AA	20	8.13	10	3.65	2.49 (1.07-6.15)	0.02
Dominant						
GG	131	53.25	163	59.40	1.0 (Reference)	-
GA+AA	115	46.75	111	40.51	1.29 (0.90-1.85)	0.15
Recessive						
GG+GA	226	91.87	264	96.35	1.0 (Reference)	-
AA	20	8.13	10	3.65	2.34 (1.02-5.70)	0.03
CYP4502E1						
Codominant model						
C1/C1	121	49.19	156	56.93	1.0 (Reference)	-
C1/C2	97	39.43	111	40.51	1.13 (0.77-1.64)	0.52
C2/C2	28	11.38	7	2.55	5.16 (2.10-14.40)	<0.001
Dominant						
C1/C1	126	51.40	156	57.10	1.0 (Reference)	-
C1/C2+C2/C2	125	50.81	118	43.07	1.31 (0.92-1.88)	0.12
Recessive						
C1/C1+C1/C2	218	88.62	267	97.45	1.0 (Reference)	-
C2/C2	28	11.38	7	2.55	4.90 (2.04-13.51)	<0.001

[1]Adjusted for smoking habit, drinking habit, family history of cancer and pickled food intake.

In the codominant model, the AA genotype of *ALDH2* Glu487Lys significantly elevated the risk of gastric cancer in comparison with the GG genotype. In the recessive model, the AA genotype of *ALDH2* Glu487Lys significantly increased the risk of gastric cancer compared to the GG+GA genotype (OR = 2.34 95%CI = 1.02-5.70). We found in the codominant model that individuals harboring the C2/C2 genotype of *CYP4502E1* had a higher risk of developing gastric cancer than those with the C1/C1 genotype. In addition, in the recessive model, we found that the C2/C2 genotype of *CYP4502E1* correlated with an elevated risk of gastric cancer in comparison with the C1/C1+C1/C2 genotype (OR = 4.90, 95%CI = 2.04-13.51).

However, the *ADH1B* Arg47His polymorphism did not affect the risk of developing gastric cancer in all the genetic models studied.

Spearman's correlation analysis indicated that the *CYP4502E1* polymorphism showed a significant association with the drinking habit, but the smoking habit, family history of cancer and pickled food intake did not reveal a significant correlation with the *CYP4502E1* polymorphism in the risk of gastric cancer (Table 4).

Table 4. Interaction between *CYP4502E1* polymorphism and confounding risk factors in the risk of gastric cancer.

Variables	Correlation coefficient	P value
Smoking habit	0.072	0.32
Drinking habit	0.250	0.02
Family history of cancer	0.092	0.25
Pickled food intake	0.046	0.56

DISCUSSION

In the present study, we analyze the association of *ADH1B* Arg47His, *ALDH2* Glu487Lys and *CYP4502E1* polymorphisms with the risk of gastric cancer, observing that the C2/C2 genotype of *CYP4502E1* was related to an increased susceptibility to this cancer in codominant and recessive models when compared with the wide-type genotype, and *CYP4502E1* polymorphism showed a significant association with the drinking habit in the risk of gastric cancer.

Acetaldehyde is a well-known carcinogen and its plasma level is determined by two enzymes, ADH1B and ALDH2. The *ADH1B* Arg47His AA genotype results in greater enzyme activity than the GA and GG genotypes, as does the *ALDH2* Glu487Lys GG genotype, in comparison to the AA and GA sequence variants (Li et al., 2008; Tseng et al., 2009). In individuals carrying the AA genotypes of both polymorphisms, plasma levels of acetaldehyde are relatively high and this compound persists in the body over long periods. Such individuals are therefore at an increased risk of developing cancer in comparison to those carrying wild-type sequences (Nishiyori et al., 2005; Yang et al., 2005; Wang et al., 2011). *CYP4502E1* encodes an important phase I biological metabolism enzyme (dimethylnitrosamine-demethylase) that can activate the nitrosamines and anilines in the human body. *CYP4502E1* is greatly induced by ethanol (Romani, 2015). CYP4502E1 is an important metabolism enzyme for removing nitro and alkyl groups, and causes the bioactivation of precarcinogens. Therefore, the different expression of *CYP4502E1* could influence the hereditary susceptibility to environmental carcinogens (Brady et al., 2002). The *CYP4502E1* polymorphisms are located at the transcriptional regulation regions, and their genetic variations could influence the expression of this protein (Hrycay and Bandiera, 2015; Wahlang et al., 2015).

Currently, it is reported that polymorphisms in *ADH1B* and *ALDH2* could change the expression of *ADH* and *ALDH*, thus modifying their enzymatic activities. It has been shown that the AA genotype of *ADH1B* is twenty times more efficient in alcohol oxidation as compared to the GG genotype (Bosron and Li, 1986). The AA and GA genotypes of *ALDH2* have 5 and 17% of the enzymatic activity of the GG genotype (Mizoi et al., 1994). Currently, several studies have reported the association of *ADH1B* Arg47His and *ALDH2* Glu487Lys polymorphisms with the risk of gastric cancer in Japanese and Chinese populations: Cao et al. (2010) performed a study with 382 patients with stomach cancer and 382 healthy controls.

They reported that ADH1B and ALDH2 polymorphisms did not contribute to gastric cancer susceptibility. Duell et al. (2012) performed an investigation of 364 gastric cancer cases and 1272 controls, and they reported that ADH1B and ALDH2 polymorphisms could influence the risk of gastric cancer, and alcohol intake could affect the role of *ADH1B* Arg47His in the risk of gastric cancer. Hidaka et al. (2015) performed an investigation with 457 new gastric cancer cases and 457 controls in Japan, and they reported that an ALDH2 polymorphism was associated with alcohol consumption in the risk of gastric cancer. Wang et al. (2014) conducted a meta-analysis with seven case-control studies (2563 patients and 4192 controls), and reported that ALDH2 and ADH1 polymorphisms may have an essential role in the development of gastric cancer. In our study, we observed that the *ALDH2* Glu487Lys polymorphism correlated with a higher risk of gastric cancer in both codominant and recessive models.

Currently, only one previous study reported the correlation between *CYP4502E1* polymorphism and the risk of cancer - in this case, esophageal cancer in a Kazakh population (Qin et al., 2008). They carried out a 1:2 matched case-control study with 120 esophageal cancer cases and 240 hospital-based controls, and they found that *CYP4502E1* polymorphism could influence the development of esophageal cancer. In our study, we first demonstrated the association of *CYP4502E1* polymorphism with the risk of gastric cancer in a Chinese population. Further studies are greatly needed to confirm this.

There are two limitations to the present study. First, the gastric cancer patients and control subjects were selected from only one hospital, and they may not be sufficiently representative of other populations. Second, the possibility of gene-gene or SNP-SNP interactions or linkage disequilibrium between polymorphisms may have had a role in the pathogenesis of gastric cancer. Third, our study had limited statistical power due to a small sample size. Therefore, further, large-scale studies are needed to confirm our results.

In summary, the results of our study indicate that the *ALDH2* Glu487Lys and *CYP4502E1* polymorphisms are potential risk factors for the development of gastric cancer in the Chinese population, suggesting that these polymorphisms could contribute to the risk of gastric cancer.

Conflicts of interest

The authors declare no conflict of interest.

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