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Association between the pre-miR-196a2 rs11614913 polymorphism and gastric cancer susceptibility in a Chinese population

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ABSTRACT. We did a case-control study to provide a more comprehensive evaluation of the association of the pre-miR-196a2 rs11614913 polymorphism with gastric cancer. Between January 2013 and December 2014, 182 patients newly diagnosed with primary gastric cancer and 182 control subjects were recruited at Zhengzhou People's Hospital. For SNP genotyping, we used the Assay Designer 3.1 to design the primers of polymerase chain reaction. Using the chi-square test, we found that patients with gastric cancer were more likely to be alcohol drinkers ($\chi^2 = 4.4$, $P = 0.04$), to have a family history of cancer in the first relatives ($\chi^2 = 5.29$, $P = 0.02$), and to be infected with *Helicobacter pylori* ($\chi^2 = 23.39$, $P < 0.001$). A significant difference in the genotype distributions of rs11614913 was observed in our study ($\chi^2 = 6.66$, $P = 0.04$). By logistic regression analysis, we found that the *CC* genotype of rs11614913 was associated with an increased risk of gastric cancer in a codominant model (OR = 2.68, 95%CI = 1.17-6.44). By stratification analysis, we found that the *CC* genotype was associated with a strongly increased risk of gastric cancer in drinkers when compared with the *TT+TC* genotype (OR = 5.63, 95%CI = 1.54-

30.76). In conclusion, the results of our study suggest an association between the rs11614913 gene polymorphism and an elevated risk of gastric cancer, especially in drinkers.

Key words: Gastric cancer; Pre-miR-196a2; Rs11614913; Genotype

INTRODUCTION

An estimated one million new cases of gastric cancer were diagnosed in 2012 (952,000 cases, 6.8% of the total newly diagnosed cancer cases), making it the fifth most common malignancy in the world, after cancers of the lung, breast, colorectum, and prostate (IARC, 2012). Various risk factors are involved in the development of gastric cancer, including environmental factors and microbial infections (Ang and Fock, 2014; Daniyal et al., 2015; Fang et al., 2015). Although *Helicobacter pylori* infection has been identified to play an important role in the development of gastric cancer, the precise etiology of gastric cancer remains unclear (Graham and Yamaoka, 2000; Ang and Fock, 2014; Fang et al., 2015). Not all patients positive for *Helicobacter pylori* infection develop gastric cancer during their lifetime, which suggests that genetic factors contribute to the development of this cancer. Many single-nucleotide polymorphisms (SNPs) have been reported to be associated with gastric carcinogenesis (Kamangar et al., 2006; Yuzhalin, 2011).

Rs11614913 (T>C) is a common variant in pre-miR-196a2 that plays a role in the development of multiple cancer types, including gastric cancer (Peng et al., 2014; Ni et al., 2015; Nikolić et al., 2015; Qi et al., 2015; Wu et al., 2015). Several studies have evaluated the effect of this polymorphism on gastric cancer risk in different populations, but the results are inconsistent. Therefore, we did a case-control study to provide a more comprehensive evaluation of the association of this polymorphism with gastric cancer risk.

MATERIAL AND METHODS

Study population

For our hospital-based case-control designed study, we recruited 182 patients who were newly diagnosed with histopathologically confirmed primary gastric cancer at the Zhengzhou People's Hospital between January 2013 and December 2014. Patients who had primary tumors other than gastric cancer, tumors of an unknown origin, or any histopathological diagnosis other than gastric cancer were excluded.

A total of 182 controls were randomly selected from individuals who received health check-ups in our hospital, and the controls were matched for age and sex with the gastric cancer patients. Controls who had any type of digestive disease were excluded from this study, including atrophic gastritis, inflammation, hyperplasia, and intestinal metaplasia.

The demographic characteristics, including sex, age, alcohol consumption, and smoking status, were collected via a self-designed questionnaire. The clinical characteristics, including histological types and TNM stage, were collected from the medical records. *H. pylori* infection was detected by serology, histological examination, or the urea breath test. Written informed consent was obtained from each participant. The study was approved by the Institutional Research Ethics Committee of the Zhengzhou People's Hospital.

DNA extraction and genotyping

For DNA extraction, approximately 2 mL of peripheral venous blood was collected from each participant and stored at -20°C in non-anticoagulant, plexiglass tubes. Genomic DNA was extracted using the ClotBlood DNA kit (Cwbio, Beijing, China) and an ultraviolet spectrophotometer (Beckman, USA) was used to analyze the concentration and purity of the extracted DNA. For SNP genotyping, we used the Assay Designer 3.1 to design polymerase chain reaction (PCR) primers. The primers for rs11614913 were designed using Sequenom Assay Design 3.1 software. The forward and reverse primers were 5'-CCC CTT GGG TTG TGG TCC AGA TA-3' and 5'-CGA AAA GGC ACT GAT GTA ACT GGC-3', respectively. The following PCR conditions were applied: an initial denaturation at 95°C for 5 min, followed by 30 cycles of annealing at 62°C for 60 s and extension at 72°C for 60 s, and a final extension at 72°C for 10 min. The PCR products were stained with ethidium bromide and analyzed under ultraviolet light on a 2% agarose gel.

Statistical analysis

Variables of demographic characteristics are reported as frequencies and percentages of study subjects. Differences in genotype and allele frequencies between cases and controls were evaluated using the chi-square test. Hardy-Weinberg equilibrium (HWE) was used to evaluate deviations between the observed and ideal Hardy-Weinberg frequencies in controls. The role of the rs11614913 polymorphism in the development of gastric cancer was evaluated by conditional logistic regression, and the results were expressed in terms of odds ratio's (ORs) and 95% confidence intervals (CIs). All P values were two sided, and $P < 0.05$ was considered statistically significant. All statistical analyses were performed using the SPSS statistical software package, version 16.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

The demographic and clinical characteristics of patients with gastric cancer and control subjects are shown in Table 1. Based on the chi-square test, patients with gastric cancer were more likely to be alcohol drinkers ($\chi^2 = 4.4$, $P = 0.04$), have a family history of cancer in first-degree relatives ($\chi^2 = 5.29$, $P = 0.02$), and be infected with *Helicobacter pylori* ($\chi^2 = 23.39$, $P < 0.001$). However, no significant differences were found between patients and controls in terms of sex ($\chi^2 = 0.01$, $P = 0.92$), age ($\chi^2 = 0.00$, $P = 1.00$), or tobacco smoking ($\chi^2 = 0.18$, $P = 0.67$).

The genotype distribution of rs11614913 in the control group conformed to the HWE ($P = 0.27$) (Table 2). A significant difference in the genotype distributions of rs11614913 was observed in our study ($\chi^2 = 6.66$, $P = 0.04$). By logistic regression analysis, we found that the *CC* genotype of rs11614913 was associated with an increased risk of gastric cancer in a codominant model (OR = 2.68, 95%CI = 1.17-6.44). In the recessive model, the *CC* genotype was observed to be correlated with an elevated increased risk of gastric cancer when compared with the *TT*+*TC* genotype.

The association between rs11614913 polymorphism and the development of gastric cancer was stratified based on sex, age, alcohol and tobacco consumption, family history of cancer in first-degree relatives, and *Helicobacter pylori* infection (Table 3). By stratification analysis, we found that the *CC* genotype was associated with a strongly increased risk of gastric cancer in drinkers when compared with the *TT*+*TC* genotype (OR = 5.63, 95%CI = 1.54-30.76).

Table 1. Demographic characteristics of gastric cancer patients and control subjects.

Variables	Patients	%	Controls	%	χ^2-test	P value
Age (years)						
<55	103	56.59	104	57.14		
≥55	79	43.41	78	42.86	0.01	0.92
Gender						
Female	58	31.87	58	31.87		
Male	124	68.13	124	68.13	0.00	1.00
Alcohol drinkers						
Never	83	45.60	103	56.59		
Ever	99	54.40	79	43.41	4.4	0.04
Tobacco smoking						
Never	104	57.14	108	59.34		
Ever	78	42.86	74	40.66	0.18	0.67
Family history of cancer in the first relatives						
No	167	91.76	177	97.25		
Yes	15	8.24	5	2.75	5.29	0.02
Helicobacter pylori						
Negative	75	41.21	121	66.48		
Positive	107	58.79	61	33.52	23.39	<0.001
Lauren's classification						
Intestinal	78	42.86				
Diffuse	104	57.14				
TNM stage						
I-II	83	45.60				
III-IV	99	54.40				

Table 2. Association between the rs11614913 polymorphism and the development of gastric cancer.

Genotype	Patients	%	Controls	%	P for HWE	χ^2-test	P value	OR (95%CI)[1]	P value
Codominant									
TT	75	41.2	92	50.6				Ref. (1.0)	-
CT	83	45.7	79	43.2				1.29 (0.82-2.04)	0.25
CC	24	13.1	11	6.2	0.27	6.66	0.04	2.68 (1.17-6.44)	0.01
Dominant									
TT	75	41.2	92	50.6				Ref. (1.0)	-
TC+CC	107	58.8	90	49.4		3.19	0.07	1.46 (0.94-2.25)	0.07
Recessive									
TT+TC	158	86.9	171	93.8				Ref. (1.0)	-
CC	24	13.1	11	6.2		5.34	0.02	2.36 (1.07-5.51)	0.02

[1]Adjusted for gender, age, alcohol drinking, family history of cancer in the first relatives and *Helicobacter pylori* infection.

Table 3. Interaction between the rs11614913 polymorphism and demographic characteristics for gastric cancer risk.

Variables	Patients		Controls		OR (95%CI)	P value
	TT+TC	CC	TT+TC	CC		
Age (years)						
<55	88	15	97	7	2.36 (0.85-7.15)	0.07
≥55	70	9	74	4	2.38 (0.63-11.00)	0.15
Sex						
Female	49	9	55	3	3.37 (0.77-20.22)	0.07
Male	109	15	116	8	2.00 (0.75-5.65)	0.13
Alcohol drinkers						
Never	77	6	95	8	0.93 (0.25-3.19)	0.89
Ever	81	18	76	3	5.63 (1.54-30.76)	0.003
Tobacco smoking						
Never	87	17	99	9	2.15 (0.85-5.75)	0.08
Ever	71	7	72	2	3.55 (0.64-35.88)	0.10
Family history of cancer in the first relatives						
No	151	16	169	8	2.24 (0.87-6.21)	0.07
Yes	7	8	2	3	0.76 (0.05-8.98)	0.80
Helicobacter pylori						
Negative	66	9	113	8	1.93 (0.62-6.02)	0.19
Positive	92	15	58	3	3.15 (0.83-17.61)	0.07

DISCUSSION

This case-control study explored the association of the rs11614913 polymorphism in pre-miR-196a2 and gastric cancer risk. We observed that the pre-miR-196a2 rs11614913 polymorphism was associated with a significantly increased risk of gastric cancer. In addition, rs11614913 interacts with the alcohol drinking habits in contributing to the development of gastric cancer in a Chinese population.

MiR-196 plays an important role in normal development and in the pathogenesis of human disease processes such as cancer (Chen et al., 2011). The miR-196 family is comprised of miR-196a-1, miR196a-2, and miR-196b. The miR-196a-1 and miR-196a-2 genes generate the same functional mature miRNA sequence miR-196a, whereas miR-196b produces a small RNA, which differs from the sequence of miR-196a by one nucleotide (Tanzer et al., 2005). By targeting its putative targets, such as the HOX, HMGA2, and annexin A1 genes, miR-196a could play important roles in tumorigenesis (Tanzer et al., 2005). Dysregulation of miR-196 expression has been reported in multiple cancer cell lines. Mature miR-196a is overexpressed in gastric cancer tissues, suggesting it also plays a role in the development of this type of cancer (Yao et al., 2009).

Only 3 studies have suggested that pre-miR-196a-2 rs11614913 contributes to pathogenesis of human cancers (Okubo et al., 2010; Wang et al., 2013; Dikeakos et al., 2014). Okubo et al. (2010) conducted a study in a Japanese population, and found that rs11614913 was correlated with the degree of *H. pylori*-induced mononuclear cell infiltration. Wang et al. (2013) reported that the rs11614913 polymorphism is correlated with the development of gastric cancer in a Chinese population. Dikeakos et al. (2014) also reported that rs11614913 was correlated with an elevated risk of gastric cancer. However, two other studies reported that rs11614913 does not cause an increased risk of gastric cancer (Parlayan et al., 2014; Xu et al., 2015). One recent meta-analysis pooled six studies, and reported that rs11614913 did not contribute to the development of gastric cancer (Zhang et al., 2015). Here, we found that the pre-miR-196a2 rs11614913 polymorphism did contribute to an increased risk of gastric cancer. The differences of the study results might be caused by differences in genetic background and by gene-environment interactions in the etiology of gastric cancer.

Our study has several limitations. Firstly, patients and controls were selected from a single hospital, and the selected controls may not be representative of the general population. Secondly, the sample size was relatively small, which may reduce the statistical power.

In conclusion, the results of our study suggest that the rs11614913 gene polymorphism is associated with an elevated risk of gastric cancer, especially in drinkers. Future studies using larger sample sizes, and employing either similar or different analytical strategies may help to elucidate the role of rs11614913 in gastric cancer development.

Conflicts of interest

The authors declare no conflict of interest.

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