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Correlation between SNPs in CDH1 and gastric cancer in Chinese population

Abstract: Background. Many recent studies revealed that the single nucleotide polymorphisms have considerable effects on the susceptibility of cancer, such as prostate cancer, lung cancer and gastric cancer. The E-cadherin, a calcium-dependent transmembrane glycoprotein encoded by CDH1 gene, is critical for epithelial construction, intercellular adhesion and cell migration. Some associations have been reported between single nucleotide polymorphisms and gastric cancer in the Chinese population. Objective. To investigate whether the single nucleotide polymorphism in CDH1 gene is associated with the susceptibility of gastric cancer in the Chinese population. Material and methods. The genotypes of 5 known single nucleotide polymorphisms (rs33935154, rs121964871, rs121964874, rs121964875, rs121964876) were determined in 359 gastric cancer patients and 368 healthy controls. High resolution melting curve detection and sequencing analysis were used in the present study. Results. There is a statistical significance in the rs121964871 C>G polymorphism between gastric cancer patients and healthy controls (OR=1.769, 95%CI: 1.051-2.976). Elderly male individuals (>50 years of age) carrying this risk factor may be more susceptible to gastric cancer. Conclusions. The results indicated that the rs121964871 C>G polymorphism is associated with the susceptibility of gastric cancer in the Chinese population, with some age and sex-dependent tendencies observed.

Keywords: Single nucleotide polymorphism, Gastric cancer, CDH1 gene

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1 Introduction

Gastric cancer (GC) is one of the most common cancers worldwide and the leading malignancy in East Asia [1]. It can be divided into two histopathological types, differentiated and undifferentiated [2], also known as intestinal and diffuse gastric cancer [3], respectively. Although after a curative resection alone or receiving some adjuvant therapies, nearly 60% of GC patients eventually died of gastric cancer [4,5]. Nowadays, gastric cancer is still a major threat to human health with little hopeful advance [6].

E-cadherin is one of the important ingredients of cell adhesion complexes in many kinds of epithelial cells [7,8]. It is a transmembrane glycoprotein encoded by the CDH1 gene, which is located in chromosome 16q22.1. E-cadherin plays a central role in the construction of adherent type junctions by mediating calcium-dependent cellular interactions. It is known as a tumor suppressor [9], dysfunction of E-cadherin will result in the transition to an invasive

epithelial cancers in human [10]. E-cadherin is also considered to be involved in intracellular signaling in epithelial cells. The downregulation of CDH1 gene in epithelial cells is usually associated with tumor formation and differentiation [11].

Single nucleotide polymorphisms (SNP), as a genetic marker, have been proved to be related with many human phenotypic differences and susceptibility to diseases [12]. It has been reported that some single nucleotide polymorphisms are associated with many diseases. For instance, polymorphism -347G/GA in CDH1 gene was observed to be associated with the risk of papillary thyroid carcinoma [13]. The c.-23G>A in the 5，-UTR of the hOGG1 gene might increase the risk of type 2 diabetes [14].

In the present study, we conducted a case-control study in the Chinese population using an appropriate sample size to determine the role of 5 known single nucleotide polymorphisms in CDH1 gene in the risk of gastric cancer. Furthermore, we investigated the impacts of age and gender on the susceptibility of gastric cancer.

2 Materials and methods

2.1 Subjects

Gastric cancer patients, 35-80 years of age (n=359, mean age: 56.69 ± 9.51 years), were recruited from Danyang People's Hospital of Jiangsu Province, China. Sample collection was conducted from 2012 to 2014. Age/sex-matched healthy controls (n=368, mean age: 58.12 ± 10.68 years) were randomly recruited from the healthy volunteers who accepted routine health examinations in the same hospital. Those who have acute or chronic digestive system diseases or cancers were excluded.

2.2 DNA extraction

Blood samples were acquired from all patients and healthy controls, and genomic DNA was extracted from peripheral blood samples using a TIANamp Blood DNA Kit totally according to the manufacturer's instruction (Tiangen Biotech, China).

2.3 SNPs screening for CDH1 gene by high-resolution melting (HRM) and sequencing analysis

Genotyping for CDH1 gene was performed by HRM detection. The primers used for detecting 5 SNPs were showed in Table 1. PCR amplification was conducted in a volume of 10μL that contained 25 ng of genomic DNA, 0.2 pmol of each primer, 1μL of 25 mM MgCl2, 0.8μL of 2.5 mM dNTPs, 1μL of 10 × Taq buffer with (NH4)2SO4, 1μL of 10 × LC Green® PLUS (Idaho Technology), 0.4 U of Taq DNA Polymerase (Fermentas), and 0.4μL of dimethyl sulfoxide (DMSO). A thermal cycler (PTC-200) was used for PCR reactions, and the amplification process consisted of an initial denaturation step at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at proper temperature for 30 s, and extension at 72°C for 30s and with a final 10 min extension at 72°C. After PCR was completed, genotyping of the polymorphisms was performed using the High resolution melting (HRM) assay. The solution

Table 1: Primer sequence of 5 SNPs in CDH1 gene used for PCR.

SNP	Primer	Sequence 5'→3'	Product size (bp)
rs33935154	Forward	GGTGAGGGACCACTGAAGAG	277
	Reverse	AAGGGAGATGTATTGGGAGG	
rs121964871	Forward	GGCGTCTGTAGGAAGGCACA	228
	Reverse	GGCTGGCATAACTTGGGAGT	
rs121964874	Forward	AGCCTGTCGAAGCAGGATTG	209
	Reverse	GGCTGGCATAACTTGGGAGT	
rs121964875	Forward	GGTTTCGGTGAGCAGGAGGG	298
	Reverse	TCGTTTGAGCCAAGGAGGGA	
rs121964876	Forward	GGTTTCGGTGAGCAGGAGGG	298
	Reverse	TCGTTTGAGCCAAGGAGGGA	

was added into a 96 well plate (Bio-Rad) by addition of 1μL 1×LCGreen® PLUS (Idaho Technology). Mixtures were overlaid with 20μL of mineral oil (Sigma) and the plate was centrifuged (1500×g for 30s). To promote heteroduplex formation, the mixtures were subsequently denatured by heating to 95°C for 30 s and cooled to 25°C. The plate was transferred to the LightScanner® (Idaho Technology), with fluorescence data collected ranging from 70 to 95°C, as the samples melted. All heterozygotes detected from the HRM assay were finally confirmed using direct sequencing to determine the accurate genotypes. Direct sequencing of the purified PCR products was performed using an ABI BigDye® Terminator v3.1 Cycle Sequencing Kit. Analyses were performed with a 3130 Genetic Analyzer (Applied Biosystems).

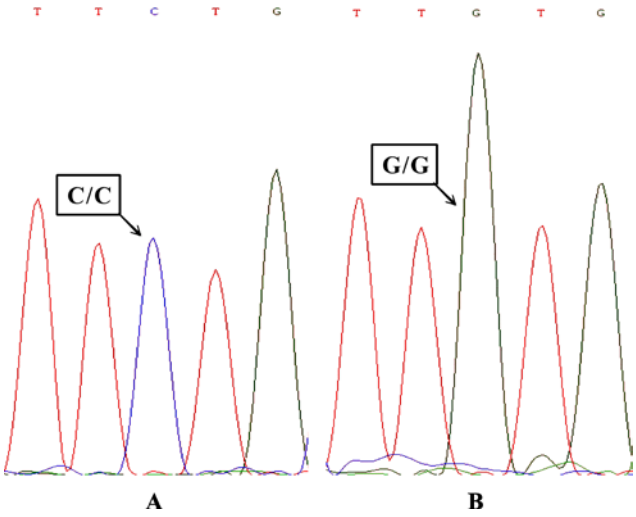

Figure 1: The single nucleotide polymorphism rs121964871 variation detection in promoter region of CDH1gene. A: homozygous wild-type, B: homozygous mutant.

2.4 Statistical analysis

Statistical analysis was carried out using the statistical program SPSS 17.0 software. Data were showed as the mean ± SD (continuous variables) and as percent totals (categorical variables). Chi-squared tests with 2×2 contingency tables were applied to compare the genotype frequencies in this case-control study, and the Student's t test was used to determine differences. The odds ratio (OR) was displayed with a 95% confidence interval (CI). Differences were considered statistically significant for a 2-tailed <0.05

2.5 Ethics

Written informed consent forms were obtained from all subjects who participated in this study. This study was approved by the Ethics Committee of Danyang People's Hospital of Jiangsu Province.

3 Results

The frequencies of genotypes for 5 single nucleotide polymorphisms of human CDH1 gene in 359 gastric cancer patients and 368 healthy controls are presented in Table 2. Three forms of PCR products for each polymorphism were analyzed by agarose gel electrophoresis, and the genotypes for each SNP were classified into three groups: take rs121964871 (C>G) as an example (Figure 1), homozygous mutant (G/G), homozygous wild-type (C/C) and heterozygous (C/G). The genotype frequencies in the controls were all in agreement with the Hardy-Weinberg equilibrium (P >0.05).

In this study, the difference in the 5 polymorphisms of CDH1 gene in human, rs121964871 (C>G) was found statistically significant (P=0.038, OR=1.769, 95%CI: 1.051-2.976) between wild-type C/C and mutants C/G + G/G in 359 gastric cancer patients and 368 healthy controls, and no significant difference was observed in other 4 SNPs (Table 2). Moreover, further stratified analysis within the rs121964871 indicated that the mutant C/G + G/G genotypes occurred at significantly higher frequency in the gastric cancer patients >50 years of age than in the parallel controls (15.22% versus 5.21%, P =0.002, OR=3.267, 95%CI: 1.538-6.937). There was no significant difference in genotype frequencies between the cancer patients in the subgroup of ≤50 years of age and the controls (Table 3). In addition, significant difference was found in genotype frequencies in the gender subgroup patients compared to the corresponding healthy controls. The mutant C/G + G/G genotypes showed significantly higher frequency in male patients than in the parallel controls (16.67% versus 8.51%, P=0.023, OR=2.150, 95%CI: 1.113-4.152), and no significant difference was observed in genotype frequencies between female cancer patients and the controls (Table 3).

4 Discussion

With the progress of urbanization, population aging and human lifestyle changes, the global prevalence of gastric cancer is rapidly increasing. It has become one of the main

Table 2: Comparison of genotypes of 5 genetic single nucleotide polymorphisms in CDH1 gene between gastric cancer patients and healthy controls.

Polymorphisms	Numbers (%)		P value [a]	OR (95%CI) [b]
	Gastric cancer (n=359)	Controls (n=368)		
rs33935154				
G/G	297 (82.7%)	321 (87.2%)		
G/A + A/A	62 (17.3%)	47 (12.8%)	0.097	
rs121964871				
C/C	318 (88.6%)	343 (93.2%)		
C/G + G/G	41 (11.4%)	25 (6.8%)	**0.038**	**1.769 (1.051-2.976)**
rs121964874				
C/C	282 (78.6%)	278 (75.5%)		
C/T + T/T	77 (21.4%)	90 (24.5%)	0.378	
rs121964875				
G/G	268 (74.7%)	296 (80.4%)		
G/A + A/A	91 (25.3%)	72 (19.6%)	0.063	
rs121964876				
G/G	305 (85%)	323 (87.8%)		
G/T + T/T	54 (15%)	45 (12.2%)	0.281	

The bold values (P<0.05) were considered statically significant.
[a] P values were analyzed for the frequencies of genotypes between gastric cancer case and control groups, P values were determined by the χ^2 test.
[b] OR (95%CI) were calculated as the wild genotypes as reference.

threats to human health in both developing and developed countries. In 2008, a total of 989,600 new gastric cancer cases and 738,000 deaths occurred in the world, accounting for 8% of total cancer cases and 10% of total deaths, and over 70% of new cases and deaths occurred in developing countries [15]. As we know, cancer is the result of the interplay between environment and genetic factors, which varies between sexes, individuals and ethnic backgrounds [16]. Gastric cancer is no exception, being affected by a combination of environmental factors and the accumulation of specific genetic alterations.

E-cadherin, encoded by CDH1 gene, is an important cell adhesion molecule and determinant of cell polarity. It is a component of the cadherin/catenin complex which is crucial for cellular polarity, differentiation and tissue morphology [17]. As a part of the adherens junction, E-cadherin offers cell-cell adhesions through Ca^{2+}-dependent bindings between molecules on adjacent epithelial cells. In most malignant tumors developed from epithelial tissues, cell-cell adhesions mediated by E-cadherin are reduced or even lost when the tumors become more malignant. Somatic loss of E-cadherin is considered to be a confirmative feature in diffuse gastric cancers [18], and pedigree mutations have been reported to predispose to hereditary diffuse gastric cancer [19].

So far, there have been many reports on the associations between single nucleotide polymorphisms in CDH1 gene and the susceptibility to gastric cancer. For instance, rs16260 C>A is a most widely studied polymorphism is CDH1 gene, and its A allele reduces the transcription efficiency of CDH1 gene and may increases the susceptibility to tumor development in some populations [20]. Higher plasma E-cadherin levels were associated with an elevated gastric cancer risk and variant genotypes of SNPs (rs26160 and rs17690554) in CDH1 gene were related to the risk of diffuse gastric cancer [21]. Some sequence alterations

Table 3: Comparison of genotypes in CDH1 rs121964871 C>G polymorphism between gastric cancer patients and healthy controls in subgroups classified by age and gender.

Groups	Genotypes	Numbers (%) Gastric cancer (n=359)	Controls (n=368)	P value [a]	OR (95%CI) [b]
Age					
≤50	C/C	162(92.57%)	161(91.48%)		
	C/G+ G/G	13(7.43%)	15(8.52%)	0.844	
>50	C/C	156(84.78%)	182(94.79%)		
	C/G+ G/G	28(15.22%)	10(5.21%)	**0.002**	**3.267(1.538-6.937)**
Gender					
Male	C/C	135(83.33%)	172(91.49%)		
	C/G+ G/G	27(16.67%)	16(8.51%)	**0.023**	**2.150(1.113-4.152)**
Female	C/C	183(92.89%)	171(95%)		
	C/G+ G/G	14(7.11%)	9(5%)	0.519	

The bold values (P<0.05) were considered statically significant.
[a] P values were analyzed for the frequencies of genotypes between gastric cancer case and control groups, P values were determined by the χ^2 test.
[b] OR (95%CI) were calculated as the wild genotype C/C as reference.

were observed in gastric cancer patients in different part of CDH1 gene, including exon, intron and promoter [22]. In the present study, 5 known single nucleotide polymorphisms were detected in a case-control study. And for the first time, rs121964871 C>G polymorphism was reported to have statistically significant difference between gastric cancer patients and healthy controls. Some related functional studies are further required to reveal the underlying possible carcinogenesis mechanisms.

In this study, some age and gender-dependent tendencies were found from further stratified analysis with rs121964871 genotypes. The individuals carrying mutants C/G or G/G genotypes occurred at significantly higher frequency in male and elderly (>50 years of age) gastric cancer patients than in the controls. On the one hand, the balance between the cell-cell adhesions mediated by E-cadherin and the protection mechanisms may be disturbed with age, leading to damages to epithelial cells and eventually causing the formation of tumor. On the other hand, our results confirmed the previous finding that gastric cancer rates are about twice as high in males as in females [15]. The specific reasons and potential mechanisms are needed to study in our further research.

In summary, we investigated the role of single nucleotide polymorphisms in CDH1 gene as the risk factor for gastric cancer in a case-control study. For the first time, we found in the Chinese population, individuals carrying variant of rs121964871 (C>G) polymorphism would be expected to be more susceptible to gastric cancer. Moreover, elderly male individuals (>50 years of age) with this polymorphism may be more likely to develop gastric cancer.

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Conflict of Interest: Authors state no conflict of interest

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