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Mutations in *CHEK2* and risk of gastric cancer: a case-control study.

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Abstract

***CHEK2* mutations have been reported to be associated with different human cancers. However, the genetic defects distribution and the roles of *CHEK2* mutations in gastric cancer carcinogenesis remain poorly understood. In this study, we detected two *CHEK2* alleles: a protein truncating (1100delC, IVS2+1G>A) and a missense variant (I157T) in 63 unselected gastric cancer cases and 96 healthy controls. The results in this study demonstrated that four SNPs (rs201688553, rs376099090, rs777046932 and rs372452522) in *CHEK2* 1100delC achieved significant difference in their distributions between gastric cancer cases and controls. Moreover, one polymorphism (rs7289973) and a novel genetic variant (IVS2-372T>C) in *CHEK2* IVS2+1G>A regions were identified and demonstrated significant difference in their distributions between gastric cancer cases and controls. Multiple logistic regression analyses revealed that gastric cancer risk were significantly associated with the variant genotypes of the four *CHEK2* polymorphisms comparing with their wild-type genotypes. Moreover, it was found that variant rs372452522 in *CHEK2* might contribute to susceptibility to lymph node metastasis. Our data demonstrated significant associations between *CHEK2* SNPs (rs201688553, rs376099090, rs777046932, rs372452522 and rs7289973) and mutation IVS2-372T>C with gastric cancer. In advance, variant *CHEK2* rs372452522 might contribute to lymph node metastasis susceptibility, implying the SNPs and mutation of *CHEK2* as potential predictors of cancer susceptibility.**

Keywords: *Cell cycle checkpoint kinase 2* (*CHEK2*), Single-nucleotide polymorphism (SNP), Gastric cancer, Polymorphisms.

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Introduction

The *CHEK2* (cell cycle checkpoint kinase 2) plays an important role in the DNA-damage signalling network and involved in cell cycle checkpoint control and apoptosis regulation Recently, several studies showed that *CHEK2* was involved in various tumor progression. For example, *CHEK2* involved breast cancer progression by repressing breast stromal fibroblasts and their paracrine tumor-promoting effects [1]. Germ line mutations or variants in the gene have been reported in cancer families or patients [2]. There are numerous *CHEK2* variants including three protein truncating alleles (1100delC, IVS2G>A, del5395) and a missense variant (I157T) [3]. Recent studies showed that *CHEK2* variants were associated with numbers of cancers including breast cancer, esophageal cancer, colon, thyroid cancers and lung cancer [2,4-8]. Mutation *CHEK2* × 1100delC, identified first in a Li-Fraumeni family with wild-type p53, is a specific variant of *CHEK2* reported with a C deleted at nucleotide position 1100 [9]. For example, a meta-analysis of 26,000 cases and 27,000 controls revealed that the *CHEK2* × 1100delC allele confers a 3-5-fold increase in risk of breast cancer, implying that *CHEK2* × 1100delC is a well-established breast cancer risk variant [10,11]. Besides, Kriege et al. showed that *CHEK2* 1100delC were associated with a higher contralateral rate as well as a worse prognosis in breast cancer [12]. I157T is a common missense variant of *CHEK2* and a recent meta-analysis with 26,336 cases and 44,219 controls from 18 case-control studies revealed significant associations of the *CHEK2* I157T variant with cancer susceptibility including breast cancer and colorectal cancer [13].

Recent studies reported that *CHEK2* is associated with gastric cancer. For example, Lee et al. revealed that aberrant expressions of *CHEK2* play a critical role in the development and progression of gastric cancer by using immunohistochemistry and tissue microarray [14]. Gutiérrez et al. reported that *CHEK2* was involved in gastric cancer chemotherapy by impairing DNA damage repair [15]. Kimura et al. also conducted related study by detecting the germ line *CHEK2* mutations in 25 familial gastric cancer cases by polymerase chain reaction-single strand conformational polymorphism analysis of the entire coding region; however they did not find germline CHK2 mutations in familial gastric

cancer cases [16]. Furthermore, Li et al analyzed the genetic variants in DNA repair pathway genes (including *CHEK2*) in gastric adenocarcinoma and it was found that the SNP in *CHEK2* is rare or not present in 1758 gastric cancers using SNPs mapping [17]. However, the relationship between *CHEK2* mutation (1100delC and I157T) and gastric cancer remains unclear.

In this study, we examined DNA samples from patients with sporadic gastric cancers for mutations in *CHEK2* (1100delC and I157T). We compared the frequency of the *CHEK2* mutations in gastric cancer groups with that in an unaffected control group thus to determine whether defects in *CHEK2* play a role in the development of gastric cancer.

Methods

Patients samples

The blood was collected from 63 primary gastric cancer patients and 96 normal healthy people from Yantai Yuhuangding Hospital from 2011 to 2016. All specimens were frozen at 80°C immediately after extraction. Patients recruited to this study did not receive any pre-operative treatments. The protocol was approved by the Research Ethics Review Board of the Yantai Yuhuangding Hospital. Informed consents were obtained from all patients. The clinical characteristics of all the patients were summarized in Table 1.

Genomic PCR and mutation analysis

DNA isolation from blood was performed following the manufacturer's protocol (QIAGEN). 13 pairs of intronic primers covering two exons of the *CHEK2* gene (1100delC and I157T) were designed. The primers thus preferentially amplified both the functional *CHEK2* on chromosome 22 and non-functional copies in the genome. PCR amplification was performed in a volume of 12.5 ml containing 25 ng of genomic DNA, according the manufacturer. Denaturing High-Performance Liquid Chromatography (DHPLC) analyses and direct sequencing of the PCR products were performed as described elsewhere [2].

Statistical analysis

Differences in the distributions of demographic characteristics, selected variables, and genotypes of the *CHEK2* variants between the cases and controls were evaluated using the $\chi 2$ test. The associations between *CHEK2* genotypes and the risk of gastric cancer were estimated by computing the ORs and their 95% CIs, using logistic regression analyses for crude ORs and adjusted ORs when adjusting for age, sex, smoking 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 ones among the control subjects. All statistical analyses were performed with SAS 9.1.3 (SAS Institute, Cary, NC).

Results

Clinicopathological features of gastric cancer

The clinicopathological features of gastric cancer cases and controls are summarized in Table 1. As shown in Table 1, there was no significant difference on the age, sex and smoking rate was detected between gastric cancer cases and controls. However, there was a statistically significant increased rate on drinking status in the cancer cases comparing with the controls (P=0.0153). However, these SNPs detected in only 10 controls people from 96 controls.

Mutations of CHEK2 with gastric cancer

In this study, we screened the *CHEK2* variant allele (1100delC) in gastric cancer patient and health people. Among the 63 cases and 96 controls with DNA samples, four SNPs (rs201688553, rs376099090, rs777046932 and rs372452522) (all four mutations combined) was observed *CHEK2* variant allele (1100delC) with 27 (42.8%) in cancer cases and 10 (10.4%) in controls. For *CHEK2* rs201688553, the genotyping was successful in 20 (31.7%) cancer cases and 5 (5.2%) controls, for *CHEK2* rs376099090 in 24 (38.1%) cancer cases and 6 (6.2%) controlsfor *CHEK2* rs777046932 in 19 (30.1%) cancer cases and 3 (3.1%) controls, and for *CHEK2* rs372452522 in 23 (36.5%) cancer cases and 4 (4.1%) controls (Figure 1).

Next, we screened the *CHEK2* variant allele (IVS2G>A) in gastric cancer cases and health controls in this study. Among the 63 cases and 96 controls with DNA samples, a novel SNP (rs7289973) was observed *CHEK2* variant allele (IVS2G>A) with 34 (53.9%) in cancer cases and 5 (5.2%) in controls (Figure 2A). Moreover, a novel mutation (IVS2-372T>C) was observed in the second exon at 372 site (Figure 2B).

Table 1. *Distribution of selected demographic variables and risk factors in gastric cancer cases and controls.*

Variable	Cases (n=63)		Controls (n=96)		P value[a]
	n	%	n	%	
Age (y)					0.556
<50	20	31.7	32	33.3	
≥ 50	43	68.3	64	66.7	
Sex					0.316
Male	38	60.3	56	58.3	
Female	25	39.7	40	41.7	
Smoking status					0.214
Never	30	47.6	51	53.1	
Ever	33	52.4	45	46.9	
Drinking status					0.013
Never	39	61.9	42	43.7	
Ever	24	38.1	54	56.3	

[a]Two-sided χ^2 test.

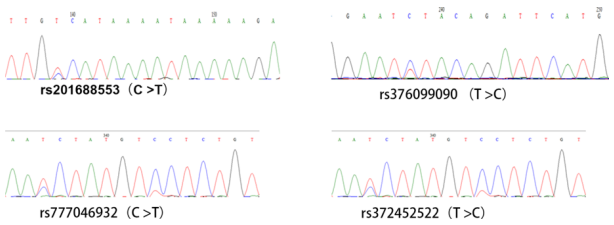

Figure 1. *Sequence analysis shows the four CHEK2 germline mutations identified in CHEK2 1100delC.*

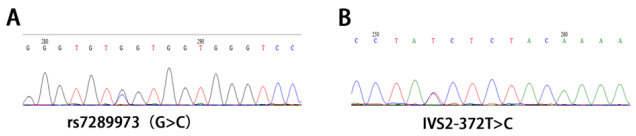

Figure 2. *Sequence analysis shows the CHEK2 germline mutations identified in VS2+1G>A regions. (A) One polymorphisms (rs7289973) in CHEK2 IVS2+1G>A regions; (B) A novel genetic variant (IVS2-372T>C) in CHEK2 IVS2+1G>A regions.*

Moreover, we detected the screened the *CHEK2* variant allele (I157T) in gastric cancer cases and health controls. However, neither SNPs nor mutations were observed in gastric cancer patients or health controls (Table 2).

Table 2. *Mutations of CHEK2 in gastric carcinoma patients and healthy controls.*

	Mutations		Cases	Control	P value	OR (95% CI)
1100delC	rs201688553	Mutation	20	5	<0.001	8.47
		Normal	43	91		(2.98~24.07)
	rs376099090	Mutation	24	6	<0.001	9.23
		Normal	39	90		(3.50~24.36)
	rs777046932	Mutation	19	3	<0.001	13.39
		Normal	44	93		(3.76~47.64)
	rs372452522	Mutation	23	4	<0.001	13.23
		Normal	40	92		(4.29~40.73)
IVS2G>A	rs7289973	Mutation	24	5	<0.001	11.2
		Normal	39	91		(3.98~31.50)

Mutations of CHEK2 with lymph node metastasis

We next analyzed the lymph node metastasis information and found that 56 of all 63 (88.8%) gastric cancer patients were observed lymph node metastasis, and 18 cancer patients (28.5%) showed regional lymph node metastasis. The observed genotype frequencies for these four polymorphisms in the controls were all in Hardy-Weinberg equilibrium (P=0.215, 0.326, 0.512 and 0.341 for *CHEK2*, including rs201688553, rs376099090, rs777046932 and rs372452522) in the controls were all in Hardy-Weinberg equilibrium (P=0.215, 0.326, 0.354, 0.421 and 0.34 respectively). In the single locus analyses, rs372452522 achieved a significant difference in the genotype distributions between the cases and the controls (P=0.0125), however, no significant difference was detected in all the three polymorphisms between the cases and controls (P=0.495, 0.5751, 0.874 for *CHEK2* rs201688553, rs376099090 and rs777046932 respectively).

Discussion

Gastric Cancer (GC) is the fourth most frequently occurring aggressive cancer and the second leading cause of death from cancer worldwide, particularly in East Asia. Both inherited genetic alterations and environmental factors may contribute to GC development. Despite of the rapid advancements in the detection and therapies, the GC incidence rate is increasing and its prognosis remains poor [18]. In this study, we detected two *CHEK2* alleles (1100delC, IVS2+1G>A and I157T) in 63 independent GC cases, and then evaluated the relationship between *CHEK2* genetic variants and gastric tumorigenesis.

CHEK2, which is a key cell cycle control gene located on chromosome 22, works as a transducer of cellular responses to DNA damage and of checkpoint kinase. It was reported to play a critical role in the DNA damage signalling network. *CHEK2* recently functioned as multi-organ cancer susceptibility gene and the polymorphisms of *CHEK2* were observed in various cancers [7,19,20]. For instance, a meta-analysis of 26,000 cases and 27,000 controls revealed that the *CHEK2* × 1100delC allele confers a 3 to 5 fold increase in risk for breast cancer, implying that *CHEK2* × 1100delC is a well-established breast cancer risk variant and Kriege et al. showed that *CHEK2* 1100delC was associated with a higher incidence rate as well as worse long-term survival in breast cancer [10-12]. In a recent meta-analysis with 26,336 cases and 44,219 controls from 18 case-control studies, it was revealed that there was a significant association of the *CHEK2* I157T variant with both breast cancer and colorectal cancer susceptibility [13]. However, the relationship between *CHEK2* mutation (1100delC and I157T) and gastric cancer remains unclear. As reported by Urszula et al., it was found that a truncating mutation of *CHEK2* (1100delC, IVS2+1G>A) and a missense mutation I157T would play a role in the GC development and progression. The data of this study provided updated knowledge of *CHEK2* mutation in the GC incidence. Besides, considering that the cases included in this study were independent clinical cases, it would provide more valuable knowledge on the importance of *CHEK2* mutation function. Besides, the included cases and controls were all Chinese Han population in this study, significant risk effect of *CHEK2* in GC cases from different genetic backgrounds demonstrated the clinical and experimental importance.

In this study, we investigated the associations between *CHEK2* mutation (1100delC, IVS2+1G>A and I157T) and gastric cancer in Chinese population. It was found that a total of four polymorphisms (rs201688553rs376099090rs777046932 and rs372452522 were detected in *CHEK2* 1100delC and one polymorphism, rs7289973, and a novel genetic variant,

IVS2-372T>C, in *CHEK2* IVS2+1G>A regions achieved significant difference in their distributions between GC cases and their controls. Advanced statistical analyses revealed that GC risk were significantly associated with the variant genotypes of the four *CHEK2* polymorphisms comparing with their wild-type genotypes. Moreover, it was found that variant rs372452522 in *CHEK2* might contribute to susceptibility to lymph node metastasis. Our data supported a significant association between *CHEK2* SNPs (rs201688553, rs376099090, rs777046932, rs372452522 and rs7289973) and a novel genetic variant (IVS2-372T>C) with gastric cancer, variant *CHEK2* rs372452522 might contribute to lymph node metastasis susceptibility, implying the genetic variants of *CHEK2* as potential predictors of cancer susceptibility.

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