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.

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 germine 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 χ2 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 χ2 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%) controls for 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.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cases (n=63)</th>
<th>Controls (n=96)</th>
<th>P valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;50</td>
<td>20</td>
<td>32</td>
<td>0.556</td>
</tr>
<tr>
<td>≥50</td>
<td>43</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td>0.316</td>
</tr>
<tr>
<td>Male</td>
<td>38</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>25</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
<td>0.214</td>
</tr>
<tr>
<td>Never</td>
<td>30</td>
<td>51</td>
<td></td>
</tr>
<tr>
<td>Ever</td>
<td>33</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>Drinking status</td>
<td></td>
<td></td>
<td>0.013</td>
</tr>
<tr>
<td>Never</td>
<td>39</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>Ever</td>
<td>24</td>
<td>54</td>
<td></td>
</tr>
</tbody>
</table>
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Table 2. Mutations of CHEK2 in gastric carcinoma patients and healthy controls.

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

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 distribution 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 specifically).

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.

References


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