Original Article

The association between phospholipase C epsilon gene (PLCE1) polymorphisms and colorectal cancer risk in a Chinese Han population: a case-control study

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Abstract: Background: Heritable factors contribute to the development of colorectal cancer (CRC). We investigated the association between single nucleotide polymorphisms in phospholipase C epsilon 1 (PLCE1) and CRC susceptibility. Methods: We selected eight tag single nucleotide polymorphisms (tSNPs) and investigated whether they were associated with CRC in Chinese Han population. In this study, we used Sequenom MassARRAY technology and genotyped 276 CRC cases and 385 controls. The effects of the polymorphisms on the risk of CRC were expressed as odds ratios (ORs) with 95% confidence intervals (95% CIs), evaluated by different genetic models using unconditional logistic regression analysis adjusted for age and gender. We also analyzed the risk of the eight PLCE1 tSNPs in different histology of CRC. Results: Based on x² tests, rs753724 (OR = 1.49, 95% CI: 1.10-2.03, P = 0.010) and rs10882424 (OR = 1.32, 95% CI: 1.02-1.70, P = 0.037) in PLCE1 were associated with CRC. In genetic model analyses, we found that rs753724 in PLCE1 may increase CRC risk (OR = 1.48, 95% CI: 1.09-2.03, P = 0.013) in the log-additive model, and rs11187842 in PLCE1 may increase CRC risk (OR = 3.09, 95% CI: 1.17-8.14, P = 0.018) in the recessive model. Rs753724 TT (OR = 4.31, P = 0.010), rs11187842 TT (OR = 5.78, P = 0.003), and rs10882424 GG (OR = 2.64, P = 0.022) in PLCE1 may increase rectal cancer in a recessive model. Conclusions: Our results suggest that PLCE1 may be associated with CRC in Han Chinese population.

Keywords: Phospholipase C epsilon 1, single nucleotide polymorphism, colorectal cancer, case-control

Introduction

Colorectal cancer (CRC), which includes colon and rectal cancers, is the third most commonly diagnosed cancer in males and the second in females [1, 2]. Although CRC is primarily a disease of high-income countries, there has been a rapid increase in CRC rates in low and middle income countries that have recently transitioned from a relatively low- or middle-income economy, such as Japan, Singapore, and countries in eastern Europe [3]. It has been recognized that CRC is a multifactorial disease caused by complex interactions between environmental and genetic factors [4]. Risk factors for CRC consist of high fat and alcohol intake, obesity, smoking, and lack of physical exercise [5]. Currently, many candidate genes have been identified, including phospholipase C epsilon 1 (PLCE1), which may be implicated in the genesis of CRC [6].

Phospholipase C epsilon 1 (PLCE1) is encoded by the PLCE1 gene on chromosome 10q23 and belongs to the phosphoinositide-specific phospholipase C (PLC) enzyme family. It contains one cell division cycle 25 domain at the N-terminus and two Ras-associating domains at the C-terminus [7, 8]. PLCE1 catalyzes the hydrolysis of polyphosphoinositides such as phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P2) to generate the second mes-
sengers Ins(1,4,5)P3 and diacylglycerol. These products initiate a cascade of intracellular responses that result in cell growth and differentiation and gene expression [6]. In addition, PLCE1 activates the small G protein Ras/mitogen-activated protein kinase (MAPK) signaling [9]. Studies have reported that PLCE1 has a fundamental role in carcinogenesis and the progression of various human cancers, including cancers of the intestine, skin, bladder, and head and neck [10-13]. However, no associations between the potentially functional variants of PLCE1 and CRC susceptibility have been reported.

Therefore, we performed genotyping analyses of eight tSNPs in PLCE1 in Chinese populations with a high incidence of CRC and assessed their associations with CRC risk in our case-control study of 276 CRC cases and 385 healthy controls. In addition, we assessed the risk of the eight PLCE1 tSNPs in different histology of CRC.

Materials and methods

Ethics statement

The use of human tissue and the protocol in this study were strictly conformed to the principles expressed in the Declaration of Helsinki and were approved by the Ethical Committee of Yulin first hospital and the Fourth Hospital of Yulin for approval of research involving human subjects. Signed informed consent was obtained from each participant.

Study population

We conducted a case-control study to assess genetic associations with CRC risk. Patients with newly diagnosed CRC from Yulin first hospital and the Fourth Hospital of Yulin, were enrolled in this study. Control subjects were randomly recruited from health centers of the two hospitals during the same period, and were matched with CRC patients based on age and gender. All cases had histologically confirmed CRC, and controls were newly-diagnosed incident patients. We excluded subjects who underwent radiotherapy or chemotherapy as well as controls with chronic disease. All participants were at least 18 years old and in good mental health. Additionally, all participants were Han Chinese. In total, 385 controls and 276 CRC cases were recruited for this study.

Clinical data and demographic information

Demographic and personal data were collected through an in-person interview using a standardized epidemiological questionnaire, which collected data on age, sex, ethnicity, residential region, diet, education status, family history of cancer, etc. For patients, related information was also collected through consultation with treating physicians or medical chart reviews. All participants signed informed consent forms, and then 5 ml peripheral blood was drawn from each subject.

tSNP selection and genotyping

A total of eight tSNPs from the PLCE1 gene were selected for our study, each with a minor allele frequency (MAF) greater than 5% in the HapMap Chinese Han Beijing population. Genomic DNA was extracted from peripheral blood using the GoldMag Whole Blood Genomic DNA Extraction kit (GoldMag Co. Ltd. Xi’an, China) according to the manufacturer’s instructions. DNA concentrations were measured using the NanoDrop 2000 (Thermo Scientific, Waltham, MA, USA). A Sequenom MassARRAY mass spectrometry analyzer (Sequenom, San Diego, CA, USA) was used for genotyping, and data were managed using Sequenom Typer 4.0 Software (Sequenom).

Statistical analysis

Statistical analyses were performed using Microsoft Excel and SPSS 16.0 statistical pack-
PLCE1 polymorphism and colorectal cancer risk

This study included 276 CRC cases (105 females, 171 males; mean age 58.20 years) and 385 controls (188 females, 197 males; mean age 50.67 years). The characteristics of cases and controls are summarized in Table 1.

As shown in Table 2, three sites (rs10509671, rs2077218, 12219592) did not fit HWE in control subjects. Based on $X^2$ tests, rs753724 (OR = 1.49, 95% CI: 1.10-2.03, $P = 0.010$) and rs10882424 (OR = 1.32, 95% CI: 1.02-1.70, $P = 0.037$) in PLCE1 were associated with CRC in allele model.

Further model association analyses were performed using logistic tests. As shown in Table 3, rs753724 in PLCE1 may increase CRC risk (OR = 1.48, 95% CI: 1.09-2.03, $P = 0.013$) in the log-additive model, and rs11187842 may increase CRC risk (OR = 3.09, 95% CI: 1.17-8.14, $P = 0.018$) in the recessive model after adjusting for age and sex.

The TT genotype of rs753724 (OR = 4.31, $P = 0.010$), TT genotype of rs11187842 (OR = 5.78,
**Table 4.** Relationship between *PLCE1* and risk of rectum and colon disease adjusted by sex and age

<table>
<thead>
<tr>
<th>SNP</th>
<th>Model</th>
<th>Genotype</th>
<th>OR (95% CI)</th>
<th>p-value</th>
<th>Genotype</th>
<th>OR (95% CI)</th>
<th>p-value</th>
<th>Genotype</th>
<th>OR (95% CI)</th>
<th>p-value</th>
<th>Genotype</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs753724</td>
<td>Rectum</td>
<td>G/G</td>
<td>1</td>
<td>0.026*</td>
<td>G/G</td>
<td>1</td>
<td>0.098</td>
<td>G/G-G/T</td>
<td>1</td>
<td>0.010*</td>
<td>1.63 (1.08–2.46)</td>
<td>0.021*</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>G/T</td>
<td>1.26 (0.73–2.19)</td>
<td></td>
<td>G/T/T/T</td>
<td>1.54 (0.93–2.55)</td>
<td></td>
<td>T/T</td>
<td>4.31 (1.45-12.82)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>T/T</td>
<td>4.56 (1.52-13.67)</td>
<td></td>
<td>G/T/T/T</td>
<td>1.18 (0.74-1.88)</td>
<td></td>
<td>T/T</td>
<td>1.70 (0.56-5.12)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Colon</td>
<td>G/G</td>
<td>1</td>
<td>0.590</td>
<td>G/G</td>
<td>1</td>
<td>0.480</td>
<td>G/G-G/T</td>
<td>1</td>
<td>0.350</td>
<td>1.20 (0.81–1.76)</td>
<td>0.360</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>G/T</td>
<td>1.11 (0.68-1.82)</td>
<td></td>
<td>G/T/T/T</td>
<td>1.74 (0.57-5.27)</td>
<td></td>
<td>T/T</td>
<td>1.70 (0.56-5.12)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>rs11187842</td>
<td>Rectum</td>
<td>C/C</td>
<td>0.009*</td>
<td>C/C</td>
<td>1</td>
<td>0.540</td>
<td>C/C-T/C</td>
<td>1</td>
<td>0.003*</td>
<td>1.46 (0.94-2.27)</td>
<td>0.099</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>T/C</td>
<td>0.83 (0.44-1.59)</td>
<td></td>
<td>T/C-T/T</td>
<td>1.19 (0.68-2.09)</td>
<td></td>
<td>T/T</td>
<td>1.66 (1.88-17.81)</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>T/T</td>
<td>5.57 (1.80-17.28)</td>
<td></td>
<td>T/C-T/T</td>
<td>5.78 (0.68-2.09)</td>
<td></td>
<td>T/T</td>
<td>5.78 (1.88-17.81)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Colon</td>
<td>C/C</td>
<td>1</td>
<td>0.700</td>
<td>C/C</td>
<td>1</td>
<td>0.670</td>
<td>C/C-T/C</td>
<td>1</td>
<td>0.580</td>
<td>0.96 (0.63-1.49)</td>
<td>0.870</td>
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<tr>
<td></td>
<td></td>
<td>T/C</td>
<td>0.84 (0.48-1.45)</td>
<td></td>
<td>T/C-T/T</td>
<td>0.40 (0.53-1.50)</td>
<td></td>
<td>T/T</td>
<td>1.43 (0.41-4.91)</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>rs10882424</td>
<td>Rectum</td>
<td>T/T</td>
<td>1</td>
<td>0.060</td>
<td>T/T</td>
<td>1</td>
<td>0.170</td>
<td>T/T-G/T</td>
<td>1</td>
<td>0.022*</td>
<td>1.45 (1.02-2.07)</td>
<td>0.042*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G/T</td>
<td>1.17 (0.70-1.93)</td>
<td></td>
<td>G/T-G/G</td>
<td>1.39 (0.87-2.20)</td>
<td></td>
<td>G/G</td>
<td>2.64 (1.17-5.91)</td>
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<tr>
<td></td>
<td></td>
<td>G/G</td>
<td>2.78 (1.21-6.36)</td>
<td></td>
<td>G/T-G/G</td>
<td>1.19 (0.79-1.80)</td>
<td></td>
<td>G/G</td>
<td>0.94 (0.39-2.27)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Colon</td>
<td>T/T</td>
<td>1</td>
<td>0.650</td>
<td>T/T</td>
<td>1</td>
<td>0.410</td>
<td>T/T-G/T</td>
<td>1</td>
<td>0.880</td>
<td>1.11 (0.79-1.55)</td>
<td>0.540</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>G/T</td>
<td>1.22 (0.79-1.89)</td>
<td></td>
<td>G/T-G/G</td>
<td>1.19 (0.79-1.80)</td>
<td></td>
<td>G/G</td>
<td>0.94 (0.39-2.27)</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>G/G</td>
<td>1.00 (0.41-2.46)</td>
<td></td>
<td>G/T-G/G</td>
<td>1.19 (0.79-1.80)</td>
<td></td>
<td>G/G</td>
<td>0.94 (0.39-2.27)</td>
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</table>

*P* < 0.05 was considered statistically significant. * indicates statistical significance.
**PLCE1** polymorphism and colorectal cancer risk

*P* = 0.003), and GG genotype of rs10882424 (OR = 2.64, *P* = 0.022) may increase rectal cancer in a recessive model after adjusting for age and sex (Table 4).

**Discussion**

This hospital-based, case-control study investigated the association between **PLCE1** and the risk of CRC in a Han Chinese population. The current results show that the rs753724 and rs11187842 polymorphisms of **PLCE1** might contribute to CRC risk. To the best of our knowledge, this is the first report evaluating the relationship between the SNPs rs753724 and rs11187842 of **PLCE1** and the risk of CRC. Our results also demonstrate that rs753724, rs11187842, and rs10882424 polymorphisms in the **PLCE1** gene increase the risk of rectum disease, but are not related to colon disease.

**PLCE1** is a member of the phospholipase C family of proteins. **PLCE1** functions differently from the other PLC family molecules [16]. It may be related to cellular differentiation and apoptosis via its interaction with the Ras family [17, 18]. Using a gene transfection strategy, Wang et al. showed that **PLCE1** overexpression inhibited tumor cell growth, decreased colony formation, reduced cellular migration, increased apoptosis, reduced tumorigenicity, and arrested cells in the G1 phase. These findings suggest that **PLCE1** overexpression suppresses the aggressive phenotypes of tumor cells [19]. **PLCE1** was observed to regulate GEF activity of CDC25 [20] and is speculated to be a receptor for Ras GTP [21, 22]. Some molecules of the Ras family are associated with cell growth, proliferation, differentiation, and apoptosis. Moreover, several molecules that are either upstream or downstream of Ras have been reported to promote oncogenesis [23].

The SNPs rs753724 (G>T), rs11187842 (C>T), and rs10882424 (T>G) are in noncoding non-promoter positions within **PLCE1**; therefore, these SNPs are unlikely to directly influence **PLCE1** expression [24, 25]. This phenomenon may be related to sequence variations in regions of the **PLCE1** gene crucial to mRNA processing and may regulate post-transcriptional modifications, protein translation, or activity of the promoter/enhancer cluster. Additionally, two SNPs (rs753724, rs11187842) were located in the core catalytic domains of the PLCε protein, which catalyzes hydrolysis of polyphosphoinositides to generate intracellular secondary messengers, such as diacylglycerol and inositol-1,4,5 trisphosphate, to contribute to intracellular signaling [24]. **PLCE1** protein initiates a cascade of intracellular responses that result in cell growth, differentiation, and gene expression. Thus, we speculate that when the two SNPs (rs753724, rs11187842) are mutated, they result in increased expression of cancer genes and uncontrolled cell division, and thus cause cancer.

In previous studies, Duan et al. and Li et al. found no significant association between rs753724 and rs11187842, located on chromosome 10q23, with CRC in the Chinese population [26, 27]. However, our results showed that the rs753724 and rs11187842 polymorphisms of **PLCE1** might contribute to an increased risk of CRC in the Han Chinese population. In addition, the rs753724, rs11187842, and rs10882424 polymorphisms increase the risk of rectum disease, but are not significantly associated with colon disease. There are a number of reasons for these potentially conflicting data. First, our study was a hospital-based study and inherent biases regarding this type of study might lead to unreliable results. Second, our sample size is small and experimental verification of larger samples is necessary. Finally, statistical error might explain these conflicting results.

**Conclusions**

Our current data show that the genotype distribution of the **PLCE1** rs753724 and rs11187842 polymorphisms differed significantly between patients with CRC and healthy control subjects. Larger population-based studies and in-depth molecular studies are needed to validate our current findings as well as to elucidate the functional roles of rs753724 and rs11187842 in CRC etiology.

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**Disclosure of conflict of interest**

None.
PLCE1 polymorphism and colorectal cancer risk

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References


PLCE1 polymorphism and colorectal cancer risk


