Association of genetic polymorphisms in PTEN and additional interaction with alcohol consumption and smoking on colorectal cancer in Chinese population

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Received August 24, 2015; Accepted October 25, 2015; Epub November 15, 2015; Published November 30, 2015

Abstract: Aims: To investigate the association of phosphatase and tensin homologue deleted on chromosome ten (PTEN) gene rs3830675, and additional interaction with drinking and smoking on colorectal cancer (CRC), based on a hospital based Chinese case-control study. Methods: A total of 850 subjects (413 males and 437 females) were studied, including 422 colorectal cancer cases and 428 controls. Rs3830675 was selected for genotyping in the case-control study. Logistic regression model was used to examine the association between rs3830675 and colorectal cancer, and additional interaction with alcohol consumption and smoking. Results: The frequencies for rs3830675 (-) alleles was higher in cases than that in controls, (-) allele of rs3830675 was 24.4% in controls and 29.4% in CRC subjects (p=0.005). Logistic analysis showed that the carriers of (-) allele of rs3830675 revealed increased CRC risk than those with (+/+) genotype, adjusted OR (95% CI) was 1.35 (1.12-1.98). We found a significant interaction between alcohol consumption and rs3830675, drinkers with (-/-) or (-/+) of rs3830675 genotype have highest colorectal cancer risk, compared to never drinking subjects with (+/+) genotype, OR (95% CI) was 2.57 (1.66-3.33), after covariates adjustment. In addition, we also found that smokers with (-/-) or (-/+) of rs3830675 genotype have highest colorectal cancer risk, compared to never smokers with (+/+) genotype, OR (95% CI) was 3.01 (1.58-6.05). Conclusions: The (-) allele of rs3830675 was positively with colorectal cancer risk. There was a significant role of interaction of rs3830675 with alcohol consumption and smoking on colorectal cancer.

Keywords: Colorectal cancer, PTEN gene, interaction, SNP, drinking

Introduction

Colorectal cancer (CRC) is the third most common malignant tumor in the world, and is associated with a poor prognosis [1]. Tumor invasion and metastasis are the leading cause of death in colorectal cancer, and the incidence of colorectal cancer is rising and patients often present in the later stages [2]. Therefore identification of those factors that regulate colorectal cancer metastasis, prognosis and optimal treatment are of great significance, because the burden of this cancer was higher and higher in recent years, particular in China [3]. For CRC the major advances have involved drugs that target the epidermal growth factor receptor (EGFR) and vascular endothelial growth factor (VEGF) [4]. The prevalence of PTEN (phosphatase and tensin homologue deleted on chromosome ten) mutations in CRC has been reported to vary between 1% and 29% [5-7]. This variability in observed PTEN mutation frequencies relates to tumor genomic instability, with PTEN mutations having been described in 14-30% of CRC with microsatellite instability (MSI-H) [5, 8].

Weitz et al [9] indicated that CRC develops as a result of interactions between genetic and environmental factors over a long period of time. Recent years, although many studies [10-12] have focused on the association between PTEN gene polymorphisms and CRC risk in different populations. However, this complex interaction on CRC development was not well defined. Alcohol consumption and smoking was risk factors for CRC development, which has been suggested in many studies [13-15]. Till now, however, less study involved in the interaction...
Table 1. General characteristics of study participants in CRC cases and controls

<table>
<thead>
<tr>
<th>Variables</th>
<th>Total (n=850)</th>
<th>CRC cases group (n=422)</th>
<th>Control group (n=428)</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>52.8±11.6</td>
<td>53.1±12.3</td>
<td>51.9±12.8</td>
<td>0.164</td>
</tr>
<tr>
<td>Males N (%)</td>
<td>413 (48.6)</td>
<td>203 (48.1)</td>
<td>210 (49.1)</td>
<td>0.824</td>
</tr>
<tr>
<td>Drinking N (%)</td>
<td>335 (39.4)</td>
<td>191 (45.3)</td>
<td>144 (33.6)</td>
<td>0.0006</td>
</tr>
<tr>
<td>Smoke N (%)</td>
<td>278 (32.7)</td>
<td>162 (38.4)</td>
<td>130 (30.4)</td>
<td>0.017</td>
</tr>
<tr>
<td>High fat diet N (%)</td>
<td>300 (35.3)</td>
<td>163 (38.6)</td>
<td>137 (32.0)</td>
<td>0.052</td>
</tr>
<tr>
<td>Low fiber diet N (%)</td>
<td>375 (44.1)</td>
<td>188 (44.5)</td>
<td>187 (43.7)</td>
<td>0.868</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>81.6±9.1</td>
<td>81.1±14.3</td>
<td>82.7±15.4</td>
<td>0.117</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.4±6.5</td>
<td>22.6±6.7</td>
<td>23.2±6.2</td>
<td>0.176</td>
</tr>
</tbody>
</table>

Means ± standard deviation for age, WC, BMI; WC, waist circumference; BMI, body mass index.

between PTEN gene and alcohol consumption, so the aim of this study was to investigate PTEN gene polymorphisms and additional interaction with alcohol consumption and smoking on CRC risk in a Chinese hospital based case-control study.

Materials and methods

Subjects

This was a hospital based case-control study. And the data come from a study named “Sensitivity in methylenetetrahydrofolate reductase gene polymorphism and colorectal cancer to chemotherapy drugs fluorouracil (NO. 201403190)”. Chinese Han patients with CRC were consecutively recruited between February 2011 and September 2013 from Henan Provincial People’s Hospital, Henan province, China. A total of 850 subjects (413 males and 437 females) were studied, including 422 CRC cases and 428 controls, with a mean age of 52.8±11.6 years old. All cases were confirmed by histopathological diagnosis. Subjects who received chemotherapy or radiotherapy before surgery were excluded from this study. Healthy controls were randomly selected from a population screening program for risk factors of CRC in the same regions and 1:1 matched to cases on the basis of age (±3 years) and sex. Blood samples were collected from each participant. Detailed personal information on demographic characteristics, diet and smoking and drinking status were collected by face to face interview. At recruitment, written informed consent was obtained from each participant in the study.

Body measurements

Data on demographic information, diet, smoking and drinking information for all participants were obtained using a standard questionnaire administered by trained staffs. We defined currently alcohol consumption as more than 1 drink of any type per month or not currently drinking as less than 1 drink of any type per month [16]; Current smokers were defined as those who have smoked for at least 100 cigarettes and still smoked at the time of the interview, individuals with no history of cigarette smoking were considered as never smokers [17, 18]; Body weight, height, and waist circumference were also measured according to standardized procedures [19]. BMI was calculated as weight in kilograms divided by the square of the height in meters. Blood samples were collected in the morning after at least 8 hours of fasting. All plasma and serum samples were frozen at -80°C until laboratory testing.

Genomic DNA extraction and genotyping

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis was performed to determine the genotype of the polymorphism of rs3830675 of PTEN gene. The 403 bp fragment encompassing the absence to presence of ATCTT insertion at down-stream of exon 4 in intron 4 of PTEN gene (rs3830675) was amplified using specific primers 5’-GGGGGTGATAACAGTATCTA-3’ and 5’-CTTTATGCAATACTTTTTCCTA-3’ (Invitrogen, Carlsbad, CA, USA). A 25 μl reaction mixture including 1.25μl SNP Genotyping Assays (20×), 12.5 μL Genotyping Master Mix (2×), 20 ng DNA, and the conditions were as follows: initial denaturation for 10 min and 95°C, denaturation for 15 s and 92°C, annealing and extension for 90 s and 60°C, 50 cycles.

Statistical analysis

The mean and SD for normally distributed continuous variables, and percentages for categorical variable, were calculated and compared between case and control group participants.
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Table 2. Genotype and allele frequencies of rs3830675 between case and control group

<table>
<thead>
<tr>
<th>SNPs</th>
<th>Genotypes and Alleles</th>
<th>Frequencies N (%)</th>
<th>OR (95% CI)²</th>
<th>P-values</th>
<th>HWE test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case (n=422)</td>
<td>Control (n=428)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs3830675</td>
<td>+/+</td>
<td>18 (4.3)</td>
<td>39 (9.1)</td>
<td>1.00</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>-/+</td>
<td>139 (32.9)</td>
<td>159 (37.1)</td>
<td>1.09</td>
<td>0.041</td>
</tr>
<tr>
<td></td>
<td>+/-</td>
<td>265 (62.8)</td>
<td>230 (53.7)</td>
<td>1.87</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>-/- or -/+</td>
<td>404 (95.7)</td>
<td>389 (90.9)</td>
<td>1.35</td>
<td>0.012</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>175 (20.7)</td>
<td>237 (27.7)</td>
<td></td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>669 (79.3)</td>
<td>619 (72.3)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

²Adjusted for gender, age, high fat diet, low fiber diet, BMI and WC.

Table 3. Interaction between rs3830675 and alcohol consumption on colorectal cancer risk

<table>
<thead>
<tr>
<th>rs3830675</th>
<th>Alcohol consumption</th>
<th>OR (95% CI)²</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>+/+</td>
<td>Never</td>
<td>1.00</td>
<td>-</td>
</tr>
<tr>
<td>+/+</td>
<td>Always</td>
<td>1.26 (1.10-1.99)</td>
<td>0.002</td>
</tr>
<tr>
<td>+/- or -/+</td>
<td>Never</td>
<td>1.17 (0.99-1.81)</td>
<td>0.068</td>
</tr>
<tr>
<td>+/- or -/+</td>
<td>Always</td>
<td>2.46 (1.31-4.42)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

²Adjusted for gender, age, high fat diet, low fiber diet, smoking, BMI and WC.

The frequencies for rs3830675 (-) allele was higher in cases than that in controls, (-) allele of rs3830675 was 24.4% in controls and 29.4% in colorectal cancer subjects (P=0.005). Logistic analysis showed a significant association between genotypes of variants in rs3830675 and increased CRC risk, after adjustment for gender, age, high fat diet, low fiber diet, BMI and WC. The carriers of (-) allele of rs3830675 revealed increased CRC risk than those with (+/+ genotype, adjusted OR (95% CI) was 1.35 (1.12-1.98).

In order to obtain the odds ratios and 95% CI for the joint effects of rs3830675 and drinking or smoking on colorectal cancer risk, we conducted interaction analysis by using logistic regression. We found that drinkers with (-/-) or (-/+) of rs3830675 genotype have highest colorectal cancer risk, compared to never drinking subjects with (+/+ genotype, OR (95% CI) was 2.57 (1.66-3.33), after covariates adjustment (Table 3). In addition, we also found that smokers with (-/-) or (-/+) of rs3830675 genotype have highest colorectal cancer risk, compared to never smokers with (+/+) genotype, OR (95% CI) was 3.01 (1.58-6.05), after covariates adjustment (Table 4).

Discussion

In this study, we found that the frequencies for rs3830675 (-) alleles was higher in cases than that in controls, (-) allele of rs3830675 was 24.4% in controls and 29.4% in colorectal cancer subjects. We also found a significant association between genotypes of variants in rs3830675 and increased CRC risk, the carri-
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Table 4. Interaction between rs3830675 and smoking on colorectal cancer risk

<table>
<thead>
<tr>
<th>rs3830675</th>
<th>Smoking</th>
<th>OR (95% CI)a</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>+/+</td>
<td>Never</td>
<td>1.00</td>
<td>-</td>
</tr>
<tr>
<td>+/+</td>
<td>Always</td>
<td>1.22 (1.05-1.69)</td>
<td>0.003</td>
</tr>
<tr>
<td>-/- or -/+</td>
<td>Never</td>
<td>1.07 (0.91-1.61)</td>
<td>0.102</td>
</tr>
<tr>
<td>-/- or -/+</td>
<td>Always</td>
<td>3.01 (1.58-6.05)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

aAdjusted for gender, age, high fat diet, low fiber diet, BMI and WC, alcohol consumption.

ers of (-) allele of rs3830675 revealed increased CRC risk than those with (+/+ ) genotype. The associations between PTEN gene variation and CRC have been investigated in many studies [20-25], however, the results were not consistent. Prior to this study, no association was detected between investigated two SNPs (rs1234214 and rs2673832) of PTEN and the development of sporadic CRC in Jewish population [20]. In another population-based study, no association was also reported between the polymorphisms of four SNPs (rs926091, rs2299939, rs2248293 and rs12357281) of PTEN gene and the development of colon cancer (CC) [21]. A Northern India population study [22] indicated that PTEN gene mutation and loss of PTEN expression may provide valuable prognostic information to aid treatment strategies for CRC patients. Inactivating PTEN mutations or loss of protein expression is found in approximately 5% and 30% of sporadic colorectal cancers [23-25]. PTEN rs3830675 polymorphism with ATCTT insertion at 109 bp downstream of exon 4 in intron 4 was one of the common PTEN polymorphisms. Most recently, increasing studies investigated the association between PTEN rs3830675 and risk of various types of cancer including CRC [10]. Canbay et al [10] conducted a hospital-based case–control study, and they found that the (-/-) genotype of PTEN IVS4 gene might be associated with increased risk for development of CRC in a Turkish population. A meta-analysis [26] on association between PTEN rs3830675 and various types of cancer, they suggested that (-/-) genotype was significantly associated with increased risk of cancer especially for digestive tract cancer compared with (+/+ ) genotype. Another study [27] indicated that CRCs with a low expression or deletion of PTEN may progress towards invasion and even metastasis; thus, PTEN may have potential as a prognostic marker in human CRC.

Weitz et al [9] indicated that CRC develops as a result of interactions between genetic and environmental factors over a long period of time. But this complex interaction on CRC development was not well defined. Alcohol consumption and smoking were main environment risk factors for CRC development, which has been suggested in many studies [13-15, 28-30]. However, as far as we know, no study focused on the interaction between PTEN and alcohol consumption and smoking. Interaction analysis results indicated that drinkers or smokers with (-/-) or (-/+) of rs3830675 genotype have highest colorectal cancer risk, compared to never drinking subjects or never smokers with (+/+ ) genotype. Bongaerts et al [13] conducted a study on interaction between alcohol consumption and alcohol dehydrogenase 1C (ADH1C) genotype, and risk of colorectal cancer in the Netherlands Cohort Study on diet and cancer, however, they found no apparent evidence for the ADH1C genotype as effect modifier of the relationship between alcohol intake and CRC. The mechanism of impact of PTEN on CRC was not well studied, Deevi et al [28] indicated that PTEN may influence apoptosis in colorectal epithelium through Cdc42 signaling, thus providing a regulatory framework for both polarized growth and programmed cell death. So far, smoking has not been considered in the stratification of individuals for CRC screening [31]. Zhao et al demonstrated a clear dose-response relationship, showing that the risk of CRC increased with cigarette smoking years, the amount of cigarettes smoked per day, and smoking cigarette pack years. Although the biological mechanism behind these interactions is unknown, it is plausible that the coexistence of alcohol consumption and current smoking and rs3830675 (-/-) or (-/+) genotype contributes to the highest CRC risk, as observed in this study.

Till now, to our knowledge, this is the first study involved in the interaction of PTEN gene with alcohol consumption and smoking on CRC. How, the limitations of this study should be considered. First, although the number of study participants met the requirement for analysis, the present sample size was relatively small. Interaction analysis should be conducted for gene- gene interaction with others SNP of PTEN or other gene, also interaction with others environmental risk factors should be investigated.
in the future studies, such as diet behavior or activity and so on.

In conclusion, we found a significant association between PTEN rs3830675 (-) alleles and increased CRC risk, and we also obtained a significant interaction of rs3830675 with alcohol consumption and smoking based on this Chinese hospital based case-control study.

Acknowledgements

The writing of this paper was supported by the Henan Provincial People’s Hospital and the study named “Sensitivity in methylenetetrahydrofolate reductase gene polymorphism and colorectal cancer to chemotherapy drugs fluorouracil (No. 201403190)”. We thank all the partners and staffs who help us in the process of this study.

Disclosure of conflict of interest

None.

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References

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