Background: This case-control study aimed to analyze the association of XRCC2 polymorphisms (rs3218408 and rs3218384) with colorectal cancer (CRC) risk. The interaction of XRCC2 polymorphisms with environmental factors was investigated as well.

Material/Methods: We enrolled 147 CRC patients and 114 healthy individuals into the study. Polymerase chain reaction (PCR)-sequencing method was performed to detect rs3218408 and rs3218384 polymorphisms. Hardy-Weinberg equilibrium (HWE) was checked in the control group. Odds ratio (OR) with 95% confidence interval (CI) represented the risk of CRC. Cross-table method was used in analyzing the interaction effects.

Results: Compared to the control group, the frequency of smokers was much higher in the case group (P<0.001). A similar result was observed in drinkers (55.8% vs. 40.4%, P=0.013). Dietary habits of all subjects were investigated as well, showing that CRC patients ate fewer vegetables than did healthy controls (P<0.001). In the analysis of polymorphisms, rs3218408 appeared to be an independent risk factor of CRC (GG: OR=2.048, 95%CI=1.032–4.061; G allele: OR=1.445, 95%CI=1.019–2.049). There were 68 (76.4%) C allele carriers (rs3218384) among smokers, which was higher than the number of G allele carriers (P<0.001). A similar outcome was observed for alcohol drinkers (P=0.048), which suggests a relationship of rs3218384 with smoking and drinking. Further analysis indicated that interaction of rs3218384 with smoking increased the risk of CRC (GG and smoking: OR=3.250, 95%CI=1.235–8.556; GC+CC and smoking: OR=2.167, 95%CI=1.175–3.996).

Conclusions: We found that rs3218408 was related with increased risk of CRC, and the interaction of rs3218384 with smoking increased the risk of CRC.

MeSH Keywords: Biodegradation, Environmental • Colorectal Neoplasms, Hereditary Nonpolyposis • Genes, vif
Background

Colorectal cancer (CRC) is one of the most common cancers worldwide. Approximately 60% of the cases occur in developed countries. Incidence rates of CRC are higher in men than in women. The development of CRC results from environmental factors, such as lifestyle, diet, and environmental pollution [1–4], while inter-individual differences in susceptibility to CRC might be brought about by genetic alterations [5]. Polymorphisms in genes belonging to detoxification, metabolic, angiogenesis, and DNA repair pathways have been proved to be linked to CRC risk [6–10]. For instance, Wang et al. reported that SNPs of high-mobility group box 1 (HMG1), an angiogenesis-related gene, showed a significant association with CRC incidence in the Chinese Han population [8]. Investigating gene polymorphisms may provide a novel approach for CRC risk evaluation.

To date, few studies have focused on the combined effects of XRCC2 polymorphisms and smoking or alcohol drinking on CRC development. The present study selected loci of 2 polymorphisms of XRCC2 according to previous outcomes and analyzed the effects of genetic polymorphisms on CRC occurrence. The interaction of XRCC2 polymorphisms and smoking or alcohol drinking during the pathogenesis of CRC was researched.

Material and Methods

Study subjects

We recruited 147 CRC patients from the Oncology Department of Chinese PLA General Hospital from September 2014 to June 2016. The diagnosis of CRC was performed by 2 experienced physicians according to clinical diagnosis, MRI or CT, and histopathological examination. CRC cases caused by familial diseases were removed from the study. The patients included 78 males and 69 females. During the study period, 114 healthy controls were enrolled from the Physical Examination Department of the same hospital from which we recruited the CRC patients. The participants in the control group did not have any tumor history, family history of cancer, genetic diseases, colitis, or Crohn’s disease. The controls were frequency-matched with CRC patients by age and sex. Clinical information on each subject was recorded for further analysis. Smoking history was defined as smoking at least 1 cigarette per day for more than 6 months. Drinking history was defined as drinking more than 100 g of alcohol per week for more than 6 months.

All the subjects signed the informed consent before the study, and the study was approved by the Ethics Institute of the hospital.

XRCC2 polymorphisms detection method

We obtained 2 ml of peripheral venous blood from each subject, then anticoagulated it by sodium citrate and stored it at −20°C for later use. Proteinase K-saturated sodium chloride method was used to extract DNA. Polymerase chain reaction (PCR)-sequencing was performed to analyze XRCC2 polymorphisms (rs3218408 and rs3218384). The PCR reaction parameters were 94°C for 2 min, 35 cycles of 94°C for 15 s, 55°C for 15 s, 72°C for 25 s, and then 72°C for 3 min. The 15-μl PCR mixture was composed of 1 μl DNA sample, 10×buffer 1.5 μl, MgCl₂ (25 mM) 1.5 μl, dNTP (10 mM) 0.3 μl, each primer (10 pmol/μl) 0.25 μl, Taq polymerase (5 U/μl) 0.25 μl, and ddH₂O 9.95 μl. The primer sequence is listed in Table 1. PCR products were sequenced by Tiangen Biotech (Beijing) CO., LTD.

Previous studies have proved that the interaction of genetic polymorphisms with environmental factors may increase or inhibit the occurrence of CRC. Jing et al. concluded that PTEN rs11202607 polymorphism showed a strong interaction with tea drinking during the development of CRC [22]. MUTYH, OGG1, and FEN1 genetic mutations were reported to modify the effect of smoking on colorectal adenoma. Meanwhile, LIG3 polymorphism modified the effect of alcohol consumption [23]. Liu et al. conducted a 2-stage case-control study and found a strong relationship of LEPR polymorphism with CRC risk; the association was strengthened by family history of cancer and cigarette smoking [24].

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

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All analyses were conducted using SPSS 18.0 software. The differences in basic characteristics between the case and control groups were compared using the \( \chi^2 \) test. Hardy-Weinberg equilibrium (HWE) was confirmed in the control group with \( \chi^2 \) method. Interaction of XRCC2 polymorphisms with smoking and drinking was investigated as well. Odds ratio (OR) and 95% confidence interval (CI) represented the risk of CRC. \( P \) values less than 0.05 indicated significant differences.

**Results**

### Basic characteristics

As shown in Table 2, the average age of the case group was 57.18 years and 58.86 years in the control group. There was no obvious difference in average age between the 2 groups (\( P=0.165 \)). Education level of each subject was determined by interview and this was found not to be a risk factor for CRC (\( P=0.966 \)). The frequency of smokers was 60.5% in the case group, which was higher than that of the control group (\( P<0.001 \)). Moreover, drinkers formed a relatively greater proportion of CRC patients (55.8%) compared to that of controls (40.4%). Diet was investigated as well, and we found that the case group had more individuals eating vegetables less than 3 times a week compared to the control group (44.2% vs. 19.3%).

**XRCC2 polymorphisms predicted high risk of CRC**

In the subsequent analysis, the relationship of XRCC2 polymorphisms with CRC risk was evaluated (Table 3). Rs3218408 GG genotype was proved to be a risk factor for CRC (OR=2.048, 95%CI=1.032-4.061). G allele carriers tended to develop CRC (OR=1.445, 95%CI=1.019-2.049) more than T allele carriers.
Table 3. XRCC2 polymorphisms and CRC risk.

| SNPs     | Case (n, %) | Control (n, %) | OR (95%CI) |
|----------|-------------|----------------|------------|
| rs3218408 |             |                |            |
| TT       | 42 (28.6)   | 40 (35.1)      | Reference  |
| TG       | 62 (42.2)   | 54 (47.4)      | 1.093 (0.621–1.926) |
| GG       | 43 (29.2)   | 20 (17.5)      | 2.048 (1.032–4.061) |
| Allele   |             |                |            |
| T        | 146 (49.7)  | 134 (55.8)     | Reference  |
| G        | 148 (50.3)  | 94 (44.2)      | 1.445 (1.019–2.049) |
| rs3218384 |             |                |            |
| GG       | 57 (38.8)   | 46 (40.4)      | Reference  |
| GC       | 66 (44.9)   | 50 (43.8)      | 1.065 (0.624–1.818) |
| CC       | 24 (16.3)   | 18 (15.8)      | 1.076 (0.522–2.220) |
| Allele   |             |                |            |
| G        | 180 (61.2)  | 142 (62.3)     | Reference  |
| C        | 114 (38.8)  | 86 (37.7)      | 1.046 (0.732–1.493) |

Table 4. XRCC2 polymorphisms and patients’ characteristics.

| Characteristics | rs3218408 | rs3218384 |
|-----------------|-----------|-----------|
|                 | TT        | TG+GG     | P value | GG   | GC+CC  | P value |
| Sex             |           |           |         |      |        |         |
| Male            | 25        | 55        | 0.253   | 28   | 52     | 0.305   |
| Female          | 27        | 40        |         | 29   | 38     |         |
| Age             |           |           | 0.786   |      | 0.266  |         |
| Education level |           |           |         |      |        |         |
| None/elementary | 12        | 29        |         | 20   | 21     |         |
| Middle or high  | 18        | 40        |         | 19   | 39     |         |
| College or high | 12        | 36        |         | 18   | 30     |         |
| Smoking         |           |           | 0.200   | <0.001 |        |
| Yes             | 22        | 67        |         | 21   | 68     |         |
| No              | 20        | 38        |         | 36   | 22     |         |
| Drinking        |           |           | 0.563   | 0.048  |        |
| Yes             | 25        | 57        |         | 26   | 56     |         |
| No              | 17        | 48        |         | 31   | 34     |         |
| Vegetable consumption (times/week) | 0.187 | 0.356 |
| <3              | 16        | 49        |         | 22   | 43     |         |
| 4–6             | 14        | 25        |         | 17   | 26     |         |
| >6              | 12        | 23        |         | 18   | 21     |         |
did. No remarkable relationship of rs3218384 polymorphism with CRC risk was found in the analysis.

We also analyzed the distribution of XRCC2 polymorphisms in CRC patients (Table 4). There were 68 (76.4%) C allele carriers (rs3218384) among smokers, was which was significantly higher than the number of G allele carriers (P<0.001), and a similar outcome was observed in drinkers (P=0.048). All these results indicate associations between rs3218384 polymorphism and smoking and drinking.

**Interaction of XRCC2 polymorphisms with smoking and drinking**

Cross-table method was used to check the interaction of XRCC2 polymorphisms with smoking and drinking (Table 5). There existed a strong association between rs3218384 and smoking, and the interaction enhanced the risk of CRC. No matter what the genotype (rs3218384) was, the smokers showed higher risk of CRC (GG and smoking: OR=3.250, 95%CI=1.235-8.556; GC+CC and smoking: OR=2.167, 95%CI=1.175-3.996). There was no association of rs3218384 with drinking.

**Table 5. Interaction of XRCC2 polymorphisms with smoking and drinking.**

| Characteristics | SNPs          | Case (n, %) | Control (n, %) | OR       | 95%CI    |
|-----------------|---------------|-------------|----------------|----------|----------|
| Smoking         | rs3218384     |             |                |          |          |
| No              | GG            | 36 (24.5)   | 39 (34.2)      | Reference| –        |
|                 | GC+CC         | 22 (15.0)   | 34 (29.8)      | 0.701    | 0.347-1.414 |
| Yes             | GG            | 21 (14.3)   | 7 (6.1)        | 3.250    | 1.235-8.556 |
|                 | GC+CC         | 68 (46.2)   | 34 (29.8)      | 2.167    | 1.175-3.996 |
| Drinking        | rs3218384     |             |                |          |          |
| No              | GG            | 31 (21.1)   | 30 (26.3)      | Reference| –        |
|                 | GC+CC         | 34 (23.1)   | 38 (33.3)      | 0.866    | 0.437-1.714 |
| Yes             | GG            | 26 (17.7)   | 16 (14.0)      | 1.573    | 0.707-3.499 |
|                 | GC+CC         | 56 (38.1)   | 30 (26.3)      | 1.806    | 0.925-3.529 |

**Discussion**

The DNA repair system ensures the integrity and precision of DNA structure by repairing DNA damage. There are 5 types of repair patterns for DNA damage: mismatch repair (MMR), base excision repair (BER), nucleotide excision repair (NER), damage reverse repair (DRR), and HRR and non-homologous end-joining (NHEJ). HRR is an important pathway for repairing DSBs [25], which might be caused by endogenous or exogenous factors. Any defects in genes of the HRR pathway can block the repair of broken double strands of DNA, thus increasing the occurrence of cancers [26].

**XRCC2** is a crucial regulator in the HRR repair of DSBs and shows a pivotal function in tumor progression [13,20]. The occurrence of DSBs activates ATM and MRN complex (RAD50, NBS1, and MRE11A) that can initiate the DNA repair process [27]; thus, the response pathway is triggered. Then, CHEK2 and the breast cancer proteins BRCA1 and BRCA2 are phosphorylated, and, simultaneously, P53 is activated [28]. Subsequently, the repair of DSBs is performed by HRR or NHEJ pathway. In the HRR pathway, XRCC2 and XRCC3 are crucial for correct chromosome segregation and apoptosis response to DSBs [29]. Perfect repair of DSBs during the DNA replication course contributes to genomic stability. Failure to repair DSBs results in tumor predisposition and carcinogenesis [27,30]. Xu et al. reported that XRCC2 overexpression inhibits CRC cell apoptosis and promotes cell proliferation [31]. **MIR-7** exhibited inhibitory and promoting effects on CRC cell proliferation and apoptosis, respectively, and the function of which may be due to targeting **XRCC2** [32]. All these reports suggest that **XRCC2** is an important element in the pathogenesis of CRC.

In the subsequent research, **XRCC2** polymorphisms have attracted much attention, and were demonstrated to be related to development of cancers. **XRCC2-41657C/T** polymorphism was identified as a risk factor of CRC in a study by Li [19]. In another study, Krupa suggested that **XRCC2** Arg188His polymorphism increased CRC risk [33]. In the present study, we investigated the effects of **XRCC2 rs3218408** and **rs3218384** on risk of CRC. **XRCC2 rs3218408** polymorphism was confirmed as a risk factor of CRC in the present study, supporting by a breast cancer study [27]. Although no relation of rs3218384 with CRC risk was found, its interaction with smoking enhanced the risk of CRC.

Our study is the first to report the interaction of **XRCC2** polymorphisms with environmental factors. However, there are still
several limitations to the present study. First, the effects of gene-gene interaction on the development of CRC, which was also crucial for the occurrence of CRC, were not investigated in this research. Second, the sample size was relatively small, and only a single Chinese population was included. Third, the influence of XRCC2 polymorphisms on clinical characteristics of CRC patients was not considered in this study. The genotype of XRCC2 SNPs might be associated with tumor characteristics, such as tumor location and clinical stage. However, the associations need to be verified in further investigations. Additionally, the molecular mechanisms underlying the regulatory function of XRCC2 polymorphisms in CRC were not investigated.

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**Conclusions**

**XRCC2** rs3218408 polymorphism is an independent risk fac- tor for CRC rs3218384 is strongly associated with smoking in the pathogenesis of CRC.