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The association of the DNA repair gene *XRCC3* Thr241Met polymorphism with susceptibility to colorectal cancer in a Chinese population

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Abstract Growing evidence suggests that the Thr241Met (T241M) polymorphism in the homologous recombination repair gene *XRCC3* may alter DNA repair capacity and subsequent susceptibility to carcinogens. In a few studies of colorectal cancer (CRC), however, the results have been discrepant. A population-based nested case–control study including 140 cases and 280 cancer-free controls was conducted to evaluate the effect of *XRCC3* polymorphism, environmental exposure, and family history (FH) on the risk of CRC. The variant allele frequency was low among the ethnic Han Chinese, but we observed a significant difference between cases (6.07%) and controls (2.32%). The analytic results of the unconditional logistic regression model adjusted by age, sex, alcohol intake, cigarette smoking, and FH of cancer in first-degree relatives showed a significantly increased risk of CRC (adjusted odds ratio [OR] = 3.13, 95% confidence interval [CI]: 1.41–6.95, $P = 0.005$) as the T/M and M/M genotypes compared with the T/T genotype, which changed weakly in consideration of the subsite (adjusted OR = 4.80, 95%CI: 1.77–12.98, $P = 0.002$ in colon cancer, adjusted OR = 2.41, 95%CI: 0.93–6.25, $P = 0.071$ in rectal cancer, respectively). Combined with environmental factors such as alcohol intake and cigarette smoking, no significant interaction could be found. However, the results revealed a significant association between FH of cancer in first-degree relatives and the risk of CRC (adjusted OR = 2.24, 95%CI: 1.18–4.25, $P = 0.014$). These results also suggest that *XRCC3* T241M polymorphism and FH of cancer may be risk factors for CRC, and the *XRCC3* 241Met allele may be an effective biomarker for genetic susceptibility to CRC. Larger studies are needed to confirm our findings and identify the underlying mechanisms.

1. Introduction

The DNA repair system plays an important role in maintaining the integrity of the genome by protecting it from carcinogen-induced damage to some extent. The polymorphisms of DNA repair genes that can modify DNA repair capacity may have an effect on individual genetic susceptibility to cancer [1]. As for the high-penetrance genes, defects in mismatch repair genes have been shown to be involved in hereditary nonpolyposis colorectal cancer (HNPCC), which can explain more than 90% of the microsatellite instability or replication error (RER+) occurring in HNPCC and in about 15% of sporadic colorectal cancer (CRC) cases with RER+ [2,3]. HNPCC, however, accounts for approximately 1–5% of all CRC [3]. Polymorphisms in low-penetrance genes, which have intricate networks with environmental factors, may play a more important role in CRC carcinogenesis in the general population [4,5].

XRCC3, one of the DNA repair genes, codes for a protein participating in the homologous recombinational repair (HRR) of DNA double-strand break (DSB) repair, which is necessary for the efficient repair of DNA strand breaks and DNA cross-links, as well as for correct chromosome segregation [6]. It is a member of the Rad51 DNA repair gene family, has been shown to interact directly with HsRad51, and like Rad55 and Rad57 in yeast, it may cooperate with HsRad51 during HRR [7]. The Thr241Met (T241M) amino acid substitution due to a C18067T

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transition at exon 7 is the most frequent polymorphism in *XRCC3*, which may affect the coding enzyme's function and/or its interaction with other proteins involved in the DNA repair. The variant allele (241Met) is associated with relatively high DNA adduct levels in lymphocyte DNA, indicating relatively low DNA repair capacity [7,8]. Many studies on the association of the *XRCC3* T241M polymorphism and cancer susceptibility have been conducted [6,9–18]. The results of the few studies on CRC have been discrepant [19–21]. To identify the association of this polymorphism with the risk of CRC, we conducted a population-based nested case–control study including 140 cases and 280 cancer-free controls in a Chinese cohort.

2. Materials and methods

2.1. Study population and sample collection

The study population came from a follow-up cohort that was built in 1989, as described previously [22]. Briefly, the study subjects comprised 140 survival cases who had developed CRC between May 1, 1990 and May 1, 2002, and who were diagnosed and registered by the cancer surveillance and registry system from the cohort. In addition, 280 cancer-free controls from the same cohort were obtained by simple randomization sampling with frequency matching by age (±5 years), sex, and habitation. Dead individuals and relatives of cases were excluded. The histologic subtypes of cases included 57 colon cancer patients and 83 rectal cancer patients. All subjects were interviewed with a questionnaire including demographic characteristics, individual lifestyle, and disease history by professionally trained interviewers. To ensure the validity of the data, repeat interviews by telephone with 5% proportion among all subjects were conducted. After the interview, with each subject's permission, a total of 5 mL of blood was collected and was separated into two sections of 2mL blood with sodium citrate anticoagulation and 3 mL of blood without anticoagulation to gain blood serum. All blood samples were stored at −60°C for long-term conservation. Informed consent was obtained from all subjects, and the study was approved by the Medical Ethical Committee of Zhejiang University School of Medicine.

2.2. XRCC3 genotyping

Genomic DNA for each subject was extracted from whole blood using an improved salting out procedure. The genotype of *XRCC3* T241M was determined by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP). The genomic DNA sequence for *XRCC3* is available (GenBank accession no. AF037222). A 552–base pair (bp) PCR product was amplified using specific primers (5′-TTG GGG CCT CTT TGA GA-3′ and 5′-AAC GGC TGA GGG TCT TCT-3′) in a total reaction volume of 20 μL containing 40 ng genomic DNA, 1 unit *Taq* polymerase, and 2.0 mmol/L $Mgcl_2$ in 1× PCR buffer (Takara Biotechnology Inc., Dalian, China), 0.2 μmol/L each primer, and 200 μmol/L each dNTP. The PCR cycling conditions were as follows: 5 minutes of initial denaturation at 94°C, followed by 35 cycles of 30 seconds at 94°C, 30 seconds at 51°C, 60 seconds at 72°C, and a final extension of 7 minutes at 72°C. After PCR, the products were digested with *Nla*III (New England BioLabs, Beverly, MA) at 37°C for 7 hours and resolved on 2.5% agarose gels. The existence of a variant Met allele creates a second *Nla*III recognition site. Subjects with the wild homozygote (T/T) exhibit two fragments (239 and 313 bp), those with the heterozygote (T/M) exhibit four fragments (105, 208, 239 and 313 bp), and those with the mutant homozygote (M/M) exhibit three fragments (105, 208, and 239 bp). To ensure the accuracy of the polymorphism analysis, 10% proportion subjects were selected by a simple random sampling for repeat analysis, and the results were 100% concordant. All genotyping work was finished without knowledge of each subject's case/control status.

2.3. Statistical analysis

The chi-square test was used to compare the distribution of categorical variables such as the *XRCC3* genotype, age, sex, and so on. Adjusted and stratified odds ratios (OR) and 95% confidence intervals (CI) were estimated by unconditional logistic regression using the procedure PHREG in SAS 6.12 (SAS Institute, Cary, NC).

3. Results

The frequency distribution of age, gender, alcohol consumption, cigarette smoking, and family history (FH) in first-degree relatives of cancer, colorectal adenoma, polyps, or inflammatory bowel diseases (IBD) is summarized in Table 1. The cases and controls were well matched by age (mean ± SD, 62.77 ± 9.52 years in cases and 62.18 ± 10.30 years in controls) and sex (the same proportion for males and females), which suggests that frequency matching was adequate. In addition, there were no significant differences between the two groups in alcohol drinking, cigarette smoking, and FH of colorectal adenoma, polyps, or inflammatory bowel disease in first-degree relatives. However, a significant association was observed between FH of cancer in first-degree relatives and CRC risk (adjusted OR = 2.24, 95%CI: 1.18–4.25, $P = 0.014$).

The frequency distribution of *XRCC3* T241M genotypes among cases and controls, as well as the estimates of CRC, are presented in Table 2. The genotype distribution among controls met the Hardy–Weinberg equilibrium ($\chi^2 = 1.169$, $P = 0.558$). As shown in Table 2, a significant difference in the frequencies of *XRCC3* T241M genotypes or the 241Met allele was observed between cases and controls (11.43% of 241T/M and M/M genotype in cases and