Association of ERCC1 and ERCC2 polymorphisms with colorectal cancer risk in a Chinese population

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The ERCC1 and ERCC2 genes are important in repairing DNA damage and genomic instability, and are involved in the nucleotide excision repair pathway. We hypothesized that single nucleotide polymorphisms (SNPs) in ERCC1 and ERCC2 are associated with the risk of colorectal cancer in a Chinese population. To test this hypothesis, we genotyped four functional SNPs (ERCC1 Asn118Asn, C8092A, ERCC2 Asp312Asn, and Lys751Gln) in a case-control study with 213 colorectal cancer cases and 240 cancer-free controls. We found that the ERCC1 C8092A polymorphism AA and CA/AA variant genotypes were associated with a significantly increased risk of colorectal cancer, compared with the CC genotype (OR = 5.70, 95% CI = 1.10–5.70 for AA versus CC, and OR = 1.58, 95% CI = 1.08–2.30 for CA/AA versus CC). Furthermore, the effect appeared to be more prominent among men, smokers, drinkers, and patients with rectal cancer. However, no other SNPs were observed for any significant association with colorectal cancer risk. These results suggest that the ERCC1 C8092A polymorphism may contribute to colorectal cancer susceptibility in the Chinese population. Further large and functional studies are needed to confirm our findings.

Colorectal cancer is currently one of the most common malignant diseases and is the leading cause of mortality in the world1. During the past few decades, the incidence and mortality of colorectal cancer have been increasing rapidly in China2. The mechanism of this form of carcinogenesis is still not fully understood. Despite environmental agents such as cigarette smoking, dietary, alcohol consumption, and obesity, found to be major risk factors for colorectal cancer, only a fraction of individuals exposed to these factors develop colorectal cancer during their lifetime3–5, suggesting that genetic factors play an important role in the development of colorectal cancer.

DNA is regularly damaged by endogenous and exogenous mutagens. The DNA repair pathways play a vital role in protecting against gene mutation caused by carcinogenesis, among which the nucleotide excision repair (NER) pathway is one of the important DNA repair systems used in correcting localized small lesions and bulky DNA damage6. Excision repair cross-complementing group 1 (ERCC1) and excision repair cross-complementing group 2/xeroderma pigmentosum group D (ERCC2/XPD) both are located on chromosome 19q13.3 that participate in the key steps of NER. ERCC1 and ERCC2 are two key rate-limiting enzymes in the multistep NER process. Some studies have suggested that low ERCC1 expression is associated with increased chemotherapeutic sensitivity and thus considered a predictive marker for patients with colorectal cancer receiving combination oxaliplatin and fluorouracil chemotherapy6, while other studies indicated that genetic variants in ERCC2 were associated with the increased risk of early relapse in colorectal cancer6. Single nucleotide polymorphisms (SNPs), as important genetic biomarkers, have been reported to be related with altered gene expression and protein activity. Several SNPs of ERCC1 and ERCC2 have been identified, of which ERCC1 rs11615 and rs3212986 SNPs (Asn118Asn and C8092A) have some effects on ERCC1 mRNA expression9, whereas ERCC2 rs1799793 (Asp312Asn) and rs13181 (Lys751Gln) SNPs are associated with suboptimal DNA repair capacity10,11. Given the role of ERCC1 and ERCC2 in carcinogenesis, we hypothesized that genetic variations in the ERCC1 and ERCC2 genes may confer individual susceptibility to colorectal cancer. Here, we performed a hospital-based case-control study to investigate the association of ERCC1 and ERCC2 polymorphisms with the risk of colorectal cancer in a Chinese population.
Results

The distributions of selected variables between cases and controls are summarized in Table 1. Briefly, there was no significant differences in the distributions of age ($P = 0.534$), sex ($P = 0.743$) and smoking status ($P = 0.777$) between the cases and controls. However, colorectal cancer cases were significantly more likely to report a family history of cancer than the controls in their first-degree relatives ($P < 0.001$). Among 213 colorectal cancer cases, 109 (51.2%) had colon cancer and 104 (48.8%) had rectal cancer. Regarding tumor stage, 17, 98, 66, and 32 patients classified as stage I, II, III, and IV, respectively.

The primary information and minor allele frequencies (MAFs) of the ERCC1 and ERCC2 SNPs are summarized in Table 2. The genotype distributions in the control subjects were all in agreement with the Hardy-Weinberg equilibrium for all four SNPs ($P = 0.315$ for ERCC1 Asn118Asn, $P = 0.426$ for ERCC1 C8092A, $P = 0.060$ for ERCC2 Asp312Asn, and $P = 0.573$ for ERCC2 Lys751Gln). Furthermore, the MAFs of ERCC1 Asn118Asn, C8092A, ERCC2 Asp312Asn, and Lys751Gln is 0.268, 0.271, 0.067, and 0.081 in the HapMap-CHB database (http://hapmap.ncbi.nlm.nih.gov), respectively, which were about the same as that in our study.

As shown in Table 2, logistic regression analysis revealed that the ERCC1 C8092A AA genotype, but not the CA genotype, was associated with a significantly increased risk of colorectal cancer, compared with the CC genotype ($P = 0.037$) (OR = 2.50, 95% CI = 1.10–5.70 for AA versus CC; and OR = 1.47, 95% CI = 0.99–2.18 for CA versus CC). The ERCC1 C8092A A allele was associated with the increased risk of colorectal cancer between the cases and controls ($P = 0.012$). Furthermore, a significant increased risk of colorectal cancer was found in the combined variant genotype CA/AA compared with the CC genotype (OR = 1.58, 95% CI = 1.08–2.30). However,
no significant association was observed between variant genotype of the other three SNPs (ERCC1 Asn118Asn, ERCC2 Asp312Asn, and ERCC2 Lys751Gln) and colorectal cancer risk.

We further evaluated the effect of the ERCC1 C8092A polymorphism on colorectal cancer risk in the subgroups stratified by age, sex, and smoking status. As shown in Table 3, we found that, compared with the C8092A CC genotype, the CA/AA genotype was associated with a significantly increased risk of colorectal cancer risk among men (OR = 1.94, 95% CI = 1.16–3.23), smokers (OR = 1.69, 95% CI = 1.06–2.67), drinkers (OR = 1.62, 95% CI = 1.05–2.51), and patients with rectal cancer (OR = 1.72, 95% CI = 1.08–2.76). However, no significant gene-environment interaction was observed.

### Discussion

In this study, we investigated the associations between four common, potentially functional genetic variants (ERCC1 Asn118Asn, C8092A, ERCC2 Asp312Asn, and Lys751Gln) and the risk of colorectal cancer in a Chinese population. We found that ERCC1 C8092A seemed to be associated with increased risk of colorectal cancer. In contrast, ERCC1 Asn118Asn, ERCC2 Asp312Asn and Lys751Gln SNPs did not observe any significant association with the risk of colorectal cancer in the Chinese population.

Decreased efficiency of DNA repair is considered as a crucial role in carcinogenesis as such defects accelerate genetic instability and the rate of genetic change. As genes in the NER pathway, ERCC1 and ERCC2 are essential to the repair of DNA adducts in colorectal cancer patients, and the meta-analyses of these data found that two SNPs in ERCC1 and ERCC2 might be useful prognostic factors for assessing clinical outcomes of oxaliplatin-based chemotherapies in colorectal cancer. However, the stability and translation of the mRNA and further influence the amount of receptor protein expressed by a cell.

In fact, like other complex diseases, colorectal cancer is a complex trait caused by both genetic and environmental factors. Although our results suggest that the SNPs of ERCC2 do not directly contribute to the susceptibility to colorectal cancer, they may perhaps affect colorectal cancer risk by combining with additional polymorphisms in other genes or non-inherited risk factors. Further, a recent meta-analysis involving 22 eligible case-control studies failed to observe significant associations of these two SNPs with the risk of colorectal cancer, suggesting other genetic variants may be associated with the carcinogenesis of colorectal cancer. Although the clinical practice of ERCC1 and ERCC2 polymorphisms and colorectal cancer risk is not yet established, our results with the integration of clinical, epidemiological, and genetic data could rationalize the individualized prevention. If validated, it could be effective to select the optimal intervention according to different genotypes of the ERCC1 and ERCC2 polymorphisms that would predict who will benefit most in the general population from colorectal cancer screening.

Several limitations of this study should be addressed. Firstly, the sample size may limit the statistical power of our study, especially for subgroup analyses. Secondly, our patients were from hospitals and controls were randomly selected from the surrounding community population, therefore inherent selection bias cannot be completely excluded. Thirdly, dietary factors appear to be among the most important determinants of colorectal cancer risk. Because the diet data was not complete, we did not analyze stratification of dietary factors on colorectal cancer risk. Further studies with dietary information are needed. Finally, although we had 80% power at a 0.05 or smaller with level to detect an OR of 1.80 or greater and 0.49 or smaller with an exposure frequency of 22.5% given our current study sample size (data not shown), the sample was relatively small, which greatly decreased statistical power of the analysis.

In conclusion, our results indicate that the ERCC1 C8092A SNP may be involved in the susceptibility of colorectal cancer in the Chinese population. Future studies with larger samples and functional evaluation are warranted to validate our findings.

### Methods

**Ethics statement.** The study was approved by the institutional review board of Nanjing University of Chinese Medicine. The informed written consent was obtained from all subjects.

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**Table 3 | Stratified analyses on association between the ERCC1 C8092A polymorphism and risk of colorectal cancer**

| Characteristics | Cases (n = 213) | Controls (n = 240) | Adjusted OR (95%CI)* |
|-----------------|----------------|-------------------|---------------------|
| Age             |                |                   |                     |
| ≤60             | 49 (52)        | 53 (35)           | 1.50 (0.80–2.82)    |
| >60             | 55 (57)        | 89 (63)           | 1.52 (0.92–2.54)    |
| Sex             |                |                   |                     |
| Male            | 35 (36)        | 42 (35)           | 1.18 (0.60–2.33)    |
| Female          | 69 (73)        | 100 (63)          | 1.69 (1.06–2.67)    |
| Smoking status  |                |                   |                     |
| Never           | 29 (31)        | 28 (23)           | 1.45 (0.62–3.42)    |
| Ever            | 75 (78)        | 114 (75)          | 1.62 (1.05–2.51)    |
| Drinking status |                |                   |                     |
| Never           | 29 (31)        | 28 (23)           | 1.45 (0.62–3.42)    |
| Ever            | 75 (78)        | 114 (75)          | 1.62 (1.05–2.51)    |
| Sex             |                |                   |                     |
| Male            | 57 (64)        | 88 (52)           | 1.94 (1.16–3.23)    |
| Female          | 47 (54)        | 54 (46)           | 1.20 (0.65–2.22)    |
| Smoking status  |                |                   |                     |
| Never           | 35 (36)        | 42 (35)           | 1.18 (0.60–2.33)    |
| Ever            | 69 (73)        | 100 (63)          | 1.69 (1.06–2.67)    |
| Drinking status |                |                   |                     |
| Never           | 29 (31)        | 28 (23)           | 1.45 (0.62–3.42)    |
| Ever            | 75 (78)        | 114 (75)          | 1.62 (1.05–2.51)    |
| Tumor site      |                |                   |                     |
| Colon           | 56 (53)        | 142 (98)          | 1.48 (0.93–2.36)    |
| Rectum          | 48 (56)        | 142 (98)          | 1.72 (1.08–2.76)    |

*ORs and P-values were adjusted for age, sex, and smoking status.
Genotyping. Genomic DNA was extracted from a leukocyte pellet by traditional proteinase K digestion followed by phenol-chloroform extraction and ethanol precipitation. SNPs were genotyped by using the TaqMan allelic discrimination assay on the platform of 7900HT Real-time PCR System (Applied Biosystems, Foster City, CA). Genotyping was performed without knowing each subject’s case or control status, and two negative controls (no DNA) included in each 384-well plate were used for quality control. The genotyping results were determined by using SDS 2.3 Allelic Discrimination Software (Applied Biosystems). Genotype analysis was performed by two persons independently in a blind fashion. In addition, 10% of randomly selected samples were repeated independently to verify genotyping results.

Statistical analyses. Hardy–Weinberg equilibrium (HWE) was tested by a goodness-of-fit χ²-test to compare the observed genotype frequencies to the expected ones among the controls. Student’s t-test or χ²-test was used to evaluate differences in the distribution of characteristics of selected variables and genotypes between the cases and controls. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by unconditional logistic regression analysis with adjustment for age, sex, and cigarette smoking. The statistical power was calculated by using the PS software (http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize). All statistical analyses were performed with SPSS statistical package, version 13.0 (SPSS Inc., Chicago, IL, USA). A value of P < 0.05 was taken as significant (2 tailed).

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Additional information

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