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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 = 2.50, 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 world[1]. During the past few decades, the incidence and mortality of colorectal cancer have been increasing rapidly in China[2]. 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 lifetime[3–5], 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 damage[6,7]. 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 chemotherapy[8], while other studies indicated that genetic variants in *ERCC2* were associated with the increased risk of early relapse in colorectal cancer[9]. 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 expression[10], whereas *ERCC2* rs1799793 (Asp312Asn) and rs13181 (Lys751Gln) SNPs are associated with suboptimal DNA repair capacity[11,12]. 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.

Table 1 | Descriptive characteristics of colorectal cancer cases and controls

	Cases (n = 213)		Controls (n = 240)		*P*
Age (mean ± SD), years	59.9 ± 12.5		60.8 ± 15.6		0.534
Gender					
Male	121	56.8%	140	58.3%	0.743
Female	92	43.2%	100	41.7%	
Smoking habit					
Never	142	66.7%	163	67.9%	0.777
Ever	71	33.3%	77	32.1%	
Family history of cancer					
No	156	73.2%	221	92.1%	<0.001
Yes	57	26.8%	19	7.9%	
Tumor site					
Colon	109	51.2%			
Rectum	104	48.8%			
Tumor stages					
I	17	8.0%			
II	98	46.0%			
III	66	31.0%			
IV	32	15.0%			

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,

Table 2 | Genotype and allele frequencies of *ERCC1* and *ERCC2* polymorphisms among cases and controls and their associations with the risk of colorectal cancer

	Cases		Controls		*P*	OR (95% CI)[c]
	n	%	n	%		
ERCC1 Asn118Asn						
CC	117	54.9%	135	56.3%	0.767[a]	1.00
CT	82	38.5%	86	35.8%		1.13 (0.78–1.67)
TT	14	6.6%	19	7.9%		0.93 (0.44–1.97)
T allele		25.8%		25.8%	0.997[b]	
ERCC1 C8092A						
CC	104	48.8%	142	59.2%	0.037[a]	1.00
CA	91	42.7%	88	36.7%		1.47 (0.99–2.18)
AA	18	8.5%	10	4.1%		2.50 (1.10–5.70)
CA/AA	109	51.2%	98	40.8%	0.027[a]	1.58 (1.08–2.30)
A allele		29.8%		22.5%	0.012[b]	
ERCC2 Asp312Asn						
GG	182	85.4%	210	87.5%	0.634[a]	1.00
GA	26	12.2%	27	11.3%		1.16 (0.65–2.07)
AA	5	2.4%	3	1.2%		1.76 (0.41–7.51)
A allele		8.5%		6.9%	0.372[b]	
ERCC2 Lys751Gln						
AA	176	82.6%	201	83.8%	0.776[a]	1.00
AC	35	16.4%	38	15.8%		1.13 (0.68–1.87)
CC	2	1.0%	1	0.4%		3.10 (0.27–35.4)
C allele		9.2%		8.3%	0.662[b]	

[a]Two-sided chi-squared test for genotype distributions between cases and controls.
[b]Two-sided chi-squared test for allele frequencies between cases and controls.
[c]ORs were adjusted for age, sex, and smoking status.

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)[a]
	CC	CA/AA	CC	CA/AA	
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	57	64	88	52	1.94 (1.16–3.23)
Female	47	45	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)

[a]ORs and *P* value were adjusted for age, sex, and smoking status.

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[13,14]. As genes in the NER pathway, *ERCC1* and *ERCC2* are essential to the repair of DNA adducts in colorectal cancer[15–18]. For example, a multitude of studies have focused on the relationship between *ERCC1* and *ERCC2* polymorphisms and the prognostics for the treatment with platinum agents 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[17]. The mutations or polymorphisms of these two genes could alter DNA repair capacity, so it was biologically plausible to assume that the *ERCC1* and *ERCC2* polymorphisms might have functional significance in colorectal cancer. The present study investigated the associations of the *ERCC1* Asn118Asn, C8092A, *ERCC2* Asp312Asn and Lys751Gln polymorphisms with risk of colorectal cancer in the Chinese population. The *ERCC1* C8092A A allele appeared to be the risk allele for developing colorectal cancer, which was more frequent in cases than in controls. The precise mechanism for the positive association of the C8092A polymorphism remains unclear, as there are no direct functional data available for this polymorphism. Because the SNP is located at the 3′-untranslated region (3′-UTR) which can be regulated by regulatory proteins and microRNAs, the C8092A polymorphism in the 3′-UTR region could affect the stability and translation of the mRNA and further influence the amount of receptor protein expressed by a cell[19,20].

In fact, like other complex diseases, colorectal cancer is a complex trait caused by both genetic and environmental factors[21–23]. 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[24]. 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[25]. Because the dietary 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.

Study populations. A total of 213 colorectal cancer cases and 240 cancer-free controls were included. In brief, the cases were incident colorectal cancer patients and were consecutively recruited from the Nanjing Hospital of T.C.M and Xuzhou First People Hospital, Jiangsu, China, starting in September 2010. Controls were randomly selected from a pool of healthy volunteers who visited the general health check-up center. The cancer-free control subjects were genetically unrelated to the cases and had no individual history of cancer. After written informed consent was obtained, demographic data and environmental exposure history were obtained from the patients using a standardized face-to-face questionnaire.

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 χ^2-test to compare the observed genotype frequencies to the expected ones among the controls. Student's t-test or χ^2-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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Author contributions

Conceived and designed the experiments: N.M. and Z.W. Performed the experiments: N.M. and Z.W. Analyzed the data: L.F. and L.M. Contributed reagents/material/analysis tools: Z.Y., Q.J., L.Q. and B.J. Wrote the manuscript: N.M. and Z.W. Reference collection and data management: J.W., Q.J., H.H. and Y.Z. Statistical analyses and paper writing: N.M. and Z.W. Study design: N.M., Z.W. and B.J.

Additional information

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