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Association of single nucleotide polymorphisms of ERCC1 and XPF with colorectal cancer risk and interaction with tobacco use

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ABSTRACT

We investigated the association between polymorphisms in excision repair cross-complementation group 1 (ERCC1) (rs3212986, rs2298881 and rs11615) and xeroderma pigmentosum-complementation group F (XPF) (rs2276466 and rs6498486) and risk of colorectal cancer. A 1:1 matched case–control study was conducted. Conditional regression analysis indicated that individuals carrying the ERCC1 rs3212986 TT genotype and T allele had a marginally increased risk of colorectal cancer when compared with subjects with the GG genotype. Similarly, subjects carrying the rs11615 TT genotype and T allele had a marginally increased risk of colorectal cancer when compared with those with the CC genotype. Stratified analysis revealed that individuals with rs3212986 TT who were current or former smokers had a significantly increased risk of colorectal cancer, and a significant interaction was found between this SNP and cigarette smoking. In conclusion, our study suggests that rs3212986 and rs11615 polymorphisms are associated with risk of colorectal cancer in a Chinese population, particularly in smokers. This finding could be useful in revealing the genetic characteristics of colorectal cancer, and suggests more effective strategies for prevention and treatment.

1. Introduction

Colorectal cancer is the third most common cancer worldwide, and it is estimated that there are 663,000 new cases in males and 571,000 cases in females each year (IARC, 2008). In China, colorectal cancer is the fourth leading cause of cancer death (IARC, 2008), and incidence of the disease is increasing (Ballinger and Anggiansah, 2007; Cunningham et al., 2010). However, incidence rates of colorectal cancer in both sexes vary 10-fold among different countries, which suggest that genetic and environmental factors play a role in the pathogenesis of this cancer. Tobacco smoking, obesity, meat and alcohol consumption, and host genetic background have all been identified as factors in the development of colorectal cancer (Foulkes, 2008; Markowitz and Bertagnolli, 2009). Identification of host genetic variants that affect risk would help in understanding the mechanisms of colorectal cancer development, and might suggest new avenues of targeted therapy.

Genetic variations in DNA repair genes affect repair of bulky DNA lesions and maintenance of genomic stability, and thus cancer risk (Kaur, 2013; Q. Li et al., 2013; Y. Li et al., 2013; Mucha et al., 2013; Natukula et al., 2013). Polymorphisms in DNA repair genes involved in nuclear excision repair (NER) could alter the efficacy of DNA repair, and thus influence individual susceptibility to colorectal cancer (Mucha et al., 2013; Yousef et al., 2013). Several types of genes are known to be involved in NER, including DNA uncoiling (ERCC2 and ERCC3), incision (ERCC1-ERCC4/XPF and ERCC5/XPG), excision, elongation, and ligation. Studies on association between variants in DNA repair genes and colorectal cancer risk have gained inconsistent results (Gil et al., 2012; Liu et al., 2012; Zhang et al., 2011). The ERCC1 gene encodes a subunit of the NER complex required for the incision step of NER, which forms a heterodimer with XPF to catalyze the 5′ incision in the process of excising the DNA lesion, and thus the ERCC1 protein may have an important role in genomic instability (Wang et al., 2011). Polymorphisms in ERCC1 could influence its function and be involved in carcinogenesis (Wood, 1997). However, few studies have investigated the association between polymorphisms in ERCC1–XPF and risk of colorectal cancer.

To further assess the association between ERCC1–XPF variants and risk of colorectal cancer in a Chinese population, we conducted a

Abbreviations: miRNAs, microRNAs; ERCC1, excision repair cross-complementation group 1; XPF, xeroderma pigmentosum-complementation group F; ERCC2, excision repair cross-complementation group 2; ERCC3, excision repair cross-complementation group 3; NER, nuclear excision repair; PCR, polymerase chain reaction; CI, confidence interval; 95% OR, odds ratio; HWE, Hardy–Weinberg equilibrium; SNP, single nucleotide polymorphism; SPSS, Statistical Package for Social Science; DNA, deoxyribonucleic acid.

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Table 1
Comparison of selected characteristics of colorectal cases and controls.

Variable	Cases N = 204	%	Controls N = 204	%	χ^2	*P* value	Crude OR (95% CI)
Age (years)							
<60	84	41.18	87	42.65			1.0 (Ref.)
≥60	120	58.82	117	57.35	0.09	0.76	1.06 (0.70–1.60)
Sex							
Female	125	61.27	125	61.27			1.0 (Ref.)
Male	79	38.73	79	38.73	0	1	1.00 (0.66–1.52)
Cigarette smoking							
Never	124	60.78	148	72.55			1.0 (Ref.)
Current and former	80	39.22	62	30.39	4.21	0.04	1.54 (1.003–2.37)
Alcohol drinking							
Never	115	56.37	121	59.31			1.0 (Ref.)
Current and former	89	43.63	83	40.69	0.36	0.55	1.13 (0.75–1.70)
Annual income (RMB)							
<10,000	129	56.37	121	59.31			1.0 (Ref.)
≥10,000	75	45.10	83	40.69	3.28	0.07	0.69 (0.46–1.05)
Cancer history in first degree relatives							
No	189	92.60	203	99.51			
Yes	15	7.40	1	0.49	12.75	<0.001	16.11 (2.42–681.38)

hospital based case–control study of five common SNPs, three in ERCC1 (rs3212986, rs2298881 and rs11615) and two in XPF (rs2276466 and rs6498486).

2. Materials and methods

2.1. Subjects

Patients with colorectal cancer were recruited at Daping Hospital of the Third Military Medical University between January 2011 and December 2012. All cases were newly and histologically diagnosed with primary colorectal carcinoma by colonoscopy. Sex and age-matched (within five years) individuals were selected from those who came to the same hospital for health checkups. Controls were excluded from the study if they had a history of colorectal, gastric, or lung cancer, or a disease of the digestive system. All cases and controls signed an informed consent form before participating in the study.

A self-designed questionnaire was used to investigate the demographic characteristics of all cases and controls. Questionnaires were completed by trained interviewers who were not aware of the study hypothesis. The sociodemographic characteristics recorded included dietary habits, alcohol consumption, tobacco smoking, and other potential confounding factors. For alcohol consumption, subjects were divided into never, current, and former drinkers. Individuals who drank more than 50 g alcohol (200 ml beers or 100 ml wine) per week for 6 months or more were regarded as current drinkers, and those who had quit drinking for more than one year were regarded as former drinkers. Regarding smoking, subjects were categorized as never, current, and former smokers. Individuals who smoked 20–50 packs of cigarettes per year, or smoked more than one cigarette per day and continued for 6 months, were regarded as current smokers, and those who had quit smoking more than one year prior were former smokers.

2.2. Genotype analysis

All study participants provided 5 ml of venous blood, and their blood samples were kept at −20 °C until use, with 0.5 mg/ml ethylenediaminetetra-acetic acid used as the anticoagulant. For genotype determination, DNA was extracted from a peripheral blood sample using a TIANamp blood DNA kit (Tiangen Biotech, Beijing, China). Genotyping of ERCC1 (rs3212986, rs2298881, and rs11615) and XPF (rs2276466 and rs6498486) polymorphisms was conducted in a 384-well plate format on the Sequenom MassARRAY® Analyzer (Sequenom, San Diego, CA, USA), and polymerase chain reaction (PCR) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry were both used for genotyping. The primers for ERCC1 (rs3212986, rs2298881, and rs11615) and XPF (rs2276466 and rs6498486) polymorphisms were designed using Sequenom® Assay Design version 3.1 software. Each PCR reaction mix comprised 50 ng genomic DNA, 200 μM dNTP, 2.5 U Taq DNA polymerase (Promega, Madison, WI, USA), and 200 μM primers, in a total volume of 20 μl. The cycling program consisted of preliminary denaturation at 95 °C for 10 min to activate the Taq polymerase, followed by 45 cycles of denaturation at 95 °C for 15 s and annealing at 60 °C for 60 s, with a final extension at 72 °C for 3 min. A randomly chosen subgroup of 10% of subjects was selected for repeat analysis, and the repeat tests had 100% concordance with the original tests.

2.3. Statistical analysis

All statistical analyses were performed using the SPSS® statistical package, version 11.0 (SPSS Inc., Chicago, IL, USA) for Windows®. Continuous and categorical variables were expressed as mean ± SD and n (%) of study participants, respectively. Categorical variables of patients and controls were compared using the χ^2-test. The Hardy–Weinberg equilibriums between groups were compared using the χ^2-test. Conditional logistic regression was conducted to assess the effects of ERCC1 (rs3212986,

Table 2
Genotype characteristics of five single nucleotide polymorphisms.

	Gene	Chromosomal location	Allele	MAF[a]		HWE[b] (*P* value) in controls
				Control group	From dbSNP	
ERCC1	rs3212986	19q13.2–q13.3	G/T	0.3085	0.2925	0.53
	rs2298881		A/C	0.226	0.1928	0.29
	rs11615		C/T	0.3645	0.3627	0.54
XPF	rs2276466	16p13.3–p13.11	C/G	0.229	0.2245	0.02
	rs6498486		A/C	0.329	0.2635	0.15

[a] Minor allele frequency.
[b] Hardy–Weinberg equilibrium.

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