



# Genetic variability of *ERCC1* and *ERCC2* genes involved in the nucleotide excision repair pathway influences the treatment outcome of gastric cancer

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**ABSTRACT.** We conducted a prospective study to investigate whether *ERCC1* rs11615 and rs3212986 and *ERCC2* rs13181 and rs1799793 gene polymorphisms could serve as potential biomarkers for the prognosis of gastric cancer. Between January 2010 and December 2012, 246 patients with pathologically proven gastric cancer who were receiving platinum-based chemotherapy were recruited from the First Affiliated Hospital of Guangxi Medical University. The genotyping of the gene polymorphisms was conducted using the polymerase chain reaction coupled with restriction fragment length polymorphism. By logistic regression analysis, we found that the AA genotype of *ERCC1* rs3212986 was associated with lower rates of complete remission and partial remission following chemotherapy in gastric cancer patients, and the OR (95%CI) was 0.19 (0.06-0.60). We found that the AA genotype of

rs3212986 was correlated with higher risk of death from gastric cancer according to the Cox proportional hazards model, and the adjusted HR (95%CI) was 1.60 (0.81-3.16). However, we found no association between *ERCC1* rs11615, *ERCC2* rs13181, and *ERCC2* rs1799793 and overall survival of gastric cancer. In conclusion, the results of the present retrospective study indicate that the *ERCC1* rs3212986 gene polymorphism has a significant effect on the pharmacokinetics and treatment outcome of gastric cancer.

**Key words:** *ERCC1*; *ERCC2*; Polymorphism; Gastric cancer; Treatment outcome

## INTRODUCTION

Gastric cancer is one of the most common malignancies in the world, and its morbidity and mortality rates rank second out of all tumors (Parkin et al., 2005). Gastric cancer can be cured if tumors are discovered early and removed surgically. However, most patients are diagnosed at an advanced stage. The development of valid therapies for advanced gastric cancer is slow (Ohtsu, 2008). Currently, systemic chemotherapy is the primary means for treatment of advanced gastric cancer, but generated resistance is a major cause of chemotherapy failure. The initiation and progression of gastric cancer is a complex process that involves both genetic and environmental factors (Ludwig and Weinstein, 2005; Yang et al., 2012). Therefore, identification of genetic biomarkers that can predict the prognosis for gastric cancer patients would greatly improve individualized therapy, post-operational treatment, and follow-up strategies.

DNA repair systems, including nucleotide excision repair (NER), base excision repair, mismatch repair, and double-strand break repair, play a critical role in maintaining the stability and integrity of the genome (Lindahl and Wood, 1999; Goode et al., 2002). NER is a versatile system that monitors and repairs DNA damage, and it is induced by endogenous and exogenous factors, including therapeutic agents (de Laat et al., 1999). Therefore, alteration of NER capacity may influence the individualized clinical outcome for patients with gastric cancer. The excision repair cross-complementation group 1 (*ERCC1*) enzyme is an essential factor involved in DNA damage repair, and *ERCC2* is responsible for the damage unwinding process (Matsunaga et al., 1995; Evans et al., 1997; Enzlin and Schärer, 2002; Coin et al., 2007). We conducted a prospective study to determine whether *ERCC1* rs11615 and rs3212986, and *ERCC2* rs13181 and rs1799793 gene polymorphisms could serve as potential biomarkers for prognosis in gastric cancer.

## MATERIAL AND METHODS

### Study population

Our study consisted of 246 patients with pathologically proven gastric cancer who were receiving platinum-based chemotherapy were recruited from the First Affiliated Hospital of Guangxi Medical University between January 2010 and December 2012. The exclusion criteria of this study are patients who had a history of other malignant tumor except for gastric cancer, had received preoperative radiotherapy or chemotherapy. Ultimately, 224 patients with

gastric cancer were included in our study, and the participation rate was 91.06%.

The gastric cancer patients and control subjects were investigated by trained nurses who collected data on demographic and clinical characteristics, such as gender, age, histological classification, TNM stage, and lymphatic metastasis. All the gastric cancer patients were histopathologically confirmed and classified based on Lauren’s classification. All study subjects signed written informed consent prior to participating in this study. The protocol of this study was approved by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University. Our study was carried out according to the Declaration of Helsinki.

All gastric cancer patients underwent platinum-based chemotherapy after participating into this study. The treatment response to platinum-based chemotherapy was assessed using the World Health Organization criteria (Miller et al., 1981). The responsive to chemotherapy included complete remission (CR) and partial remission (PR), and the non-responsive to chemotherapy included stable disease and progressive disease. Overall survival (OS) was taken as the endpoint indicator. The followed-up period was between January 2010 and December 2014.

### Genotyping

A peripheral blood sample (5 mL) was drawn from the gastric cancer patients and the controls, and the blood samples were stored at -20°C. Genotyping of *ERCC1* rs11615 and rs3212986, and *ERCC2* rs13181 and rs1799793 gene polymorphisms was carried out by polymerase chain reaction (PCR) coupled with restriction fragment length polymorphism. The primer sequences for *ERCC1* rs11615 and rs3212986, and *ERCC2* rs13181 and rs1799793 were designed using the Primer premier v5.0 software (Applied Biosystems). The primers and PCR conditions are shown in Table 1. The PCR conditions were as follows: 95°C for 5 min; 30 cycles of 95°C for 30s, 60°C for 30s, and 72°C for 30s; and a final extension step of 72°C for 10 min.

**Table 1.** Methodology for polymerase chain reaction (PCR) coupled with restriction fragment length polymorphism (RFLP) analysis.

Gene	SNP	Primers	Product (bp)
<i>ERCC1</i>	rs11615	5'-GCAGAGCTCACCTGAGGAAC-3' 5'-GAGGTGCAAGAAGAGGTGGA-3'	285
<i>ERCC1</i>	rs3212986	5'-CAGAGACAGTGCCCAAGAG-3' 5'-GGGCACCTTCAGCTTCTTT-3'	380
<i>ERCC2</i>	rs13181	5'-GGCAAGACTCAGGAGTCACC-3' 5'-CCCTCTCCCTTCTCTGTT-3'	171
<i>ERCC2</i>	rs1799793	5'-CAGCTCATCTCTCCGAGGATCAA-3' 5'-GTCGGGGCTCACCTGCAGCACTTCTCT-3'	117

SNP = single-nucleotide polymorphism.

### Statistical analysis

Continuous variables are reported as means ± standard deviation and categorical variables are expressed as number (N) and percentage (%). The association between *ERCC1* rs11615 and rs3212986, and *ERCC2* rs13181 and rs1799793 and the response to chemotherapy was analyzed by logistic regression analysis, and the results are described using odds ratios (ORs) and 95% confidence intervals (95% CIs). The association between *ERCC1* rs11615 and rs3212986, and *ERCC2* rs13181 and rs1799793 with OS of gastric cancer was assessed using

multivariate analysis using the Cox proportional hazards models, and the results are described by hazard ratios (HRs) and 95% CIs. A P value less than 0.05 was considered statistically significant. Statistical analysis was carried out using the statistical package SPSS 18 software (version 18.0, SPSS Inc., Chicago, IL, USA).

## RESULTS

The median age of the gastric cancer patients was  $57.4 \pm 9.20$  years. There were 79 (35.27%) females and 145 (64.73%) males in our study. Of the 224 gastric cancer patients, 87 (38.84%) were of the intestinal type according to Lauren's classification, 137 (61.16%) were of the diffuse type, 33 (14.73%) were at the I-II TNM stage, 191 (85.27%) were at the III-IV TNM stage, 141 (62.95%) showed positive lymphatic metastasis, and 142 (63.39%) showed CR+PR response to chemotherapy (Table 2).

**Table 2.** Characteristics of patients with gastric cancer.

Characteristics	Patients with gastric cancer	%
Age (years)	$57.40 \pm 9.20$	
Gender		
Female	79	35.27
Male	145	64.73
Lauren's classification		
Intestinal	87	38.84
Diffuse	137	61.16
TNM stage		
I-II	33	14.73
III-IV	191	85.27
Lymphatic metastasis		
Negative	83	37.05
Positive	141	62.95
Response to chemotherapy		
CR + PR	142	63.39
SD + PD	82	36.61

CR = complete remission, PR = partial remission, SD = stable disease, PD = progressive disease.

The relationships between *ERCC1* rs11615 and rs3212986, and *ERCC2* rs13181 and rs1799793 gene polymorphisms and the response to chemotherapy in gastric cancer patients are shown in Table 3. By logistic regression analysis, we found that the AA genotype of *ERCC1* rs3212986 was associated with lower rates of CR + PR following chemotherapy in gastric cancer patients, and the OR (95%CI) was 0.19 (0.06-0.60). However, we did not find an association between *ERCC1* rs11615, *ERCC2* rs13181, and *ERCC2* rs1799793 and response to chemotherapy.

Up to December 2014, 63 patients died during the follow-up period, and the five-year OS rate was 71.88%. We found that the AA genotype of *ERCC1* rs3212986 was correlated with higher risk of death from gastric cancer using the Cox proportional hazards models, and the adjusted HR (95%CI) was 1.60 (0.81-3.16) (Table 4). However, no association between *ERCC1* rs11615, *ERCC2* rs13181, and *ERCC2* rs1799793 and OS in gastric cancer was found using the Cox proportional hazards models.

**Table 3.** Association between *ERCC1* and *ERCC2* gene polymorphisms and response to chemotherapy.

SNPs	Patients	%	CR + PR	%	SD + PD	%	OR (95%CI) <sup>1</sup>	P value
rs11615								
TT	108	48.21	72	50.70	36	43.90	1.0 (Ref.)	-
TC	94	41.96	58	40.85	36	43.90	0.81 (0.43-1.49)	0.46
CC	22	9.82	12	8.45	10	12.20	0.60 (0.21-1.72)	0.28
rs3212986								
CC	113	50.45	80	56.34	33	40.24	1.0 (Ref.)	-
CA	93	41.52	56	39.44	37	45.12	0.62 (0.34-1.16)	0.11
AA	19	8.48	6	4.23	13	15.85	0.19 (0.06-0.60)	<0.001
rs13181								
AA	116	51.79	77	54.23	39	47.56	1.0 (Ref.)	-
AC	84	37.50	51	35.92	33	40.24	0.78 (0.42-1.46)	0.41
CC	24	10.71	14	9.86	10	12.20	0.71 (0.27-1.96)	0.45
rs1799793								
GG	124	55.36	82	57.75	42	51.22	1.0 (Ref.)	-
GA	76	33.93	46	32.39	30	36.59	0.79 (0.42-1.48)	0.42
AA	24	10.71	14	9.86	10	12.20	0.72 (0.27-1.97)	0.46

SNP = single-nucleotide polymorphism. <sup>1</sup>Adjusted for age, gender, Lauren’s classification, TNM stage, and lymphatic metastasis.

**Table 4.** Association between *ERCC1* and *ERCC2* gene polymorphisms and overall survival of gastric cancer.

Genotypes	Patients	%	Death	%	Alive	%	HR (95%CI) <sup>1</sup>	P value
rs11615								
TT	108	48.21	29	46	79	49.07	1.0 (Ref.)	-
TC	94	41.96	27	42.8	67	41.61	1.10 (0.56-2.13)	0.77
CC	22	9.82	7	11.2	15	9.32	1.27 (0.39-3.73)	0.64
rs3212986								
CC	113	50.45	24	43.6	89	55.28	1.0 (Ref.)	-
CA	93	41.52	28	43.7	65	40.37	1.60 (0.81-3.16)	0.14
AA	19	8.48	11	12.7	8	4.97	5.10 (1.63-16.17)	<0.001
rs13181								
AA	116	51.79	31	48.5	85	52.80	1.0 (Ref.)	-
AC	84	37.5	24	38.7	60	37.27	1.10 (0.56-2.15)	0.77
CC	24	10.71	8	12.8	16	9.94	1.37 (0.46-3.80)	0.51
rs1799793								
GG	124	55.36	33	52.1	91	56.52	1.0 (Ref.)	-
GA	76	33.93	22	35.5	54	33.54	1.12 (0.56-2.22)	0.72
AA	24	10.71	8	12.4	16	9.94	1.38 (0.46-3.80)	0.5

<sup>1</sup>Adjusted for age, gender, Lauren’s classification, TNM stage, and lymphatic metastasis.

## DISCUSSION

The ERCC1 enzyme belongs to the NER system, and has the ability to repair DNA adducts and other DNA helix-distorting lesions (de Laat et al., 1999), including platinum intra-strand DNA adducts; it is thought to be involved in resistance to platinum-based chemotherapy via reducing platinum-induced DNA damage (Kwon et al., 2007; Kawashima et al., 2011). In our study, we found that the AA genotype of *ERCC1* rs3212986 was associated with the response to platinum-based chemotherapy and OS in gastric cancer patients.

The ERCC1 enzyme is involved in recognizing and removing platinum-induced intra-strand adducts in DNA; previous studies have reported that *ERCC1* gene polymorphisms are associated with the response to platinum-based chemotherapy in patients with osteosarcoma, breast cancer, non-small cell lung cancer, and colorectal cancer (Bewick et al., 2011; Qian et

al., 2014; Ji and He, 2015; Shi et al., 2015). Guo et al. (2015) conducted a study in a Canadian population, and found that the *ERCC1* rs3212986 polymorphism was not associated with the survival outcome of patients with breast cancer. Ji and He (2015) conducted a study in a Chinese population, and found that the *ERCC1* rs11615 polymorphism plays an important role in the response to chemotherapy and OS in osteosarcoma. Shi et al. (2015) also conducted a study in a Chinese population, and found that the *ERCC1* rs11615 polymorphism influences the chemotherapy and survival of patients with non-small cell lung cancer. Qian et al. (2014) reported that the *ERCC1* rs11615 polymorphism is associated with prognosis in colorectal cancer patients. The studies mentioned above suggest that *ERCC1* gene polymorphisms are associated with prognosis in cancer.

Several previous studies have reported an association between *ERCC1* gene polymorphisms and prognosis in gastric cancer (Li et al., 2014; Liu et al., 2014; Ma et al., 2015; Xue et al., 2015; Yu et al., 2015; Zhou et al., 2015). Xue et al. (2015) reported that *ERCC1* rs11615 and rs3212986 polymorphisms affect the clinical outcome of patients with gastric cancer. Another study in a Chinese population reported that *ERCC1* rs11615 and rs2298881 contribute to the response to chemotherapy and clinical outcome in gastric cancer patients (Yu et al., 2015). Zhou et al. (2015) reported that the *ERCC1* rs11615 polymorphism influences the treatment outcome in gastric cancer. Ma et al. (2015) conducted a meta-analysis comprising 22 studies, and reported that the *ERCC1* rs11615 polymorphism was correlated with prognosis in gastric cancer patients treated with platinum-based chemotherapy. In this study, we hypothesized that the AA genotype of *ERCC1* rs3212986 influences the response to platinum-based chemotherapy and OS in gastric cancer patients, which was inconsistent with our results. The inconsistency might have been due to differences in ethnicity, patient sources, sample size, and by chance.

In conclusion, the results of the present retrospective study indicate that the *ERCC1* rs3212986 gene polymorphism has a significant effect on the pharmacokinetics and treatment outcome of gastric cancer. Future studies with larger sample sizes are needed to confirm the role of *ERCC1* and *ERCC2* gene polymorphisms in the treatment outcome for gastric cancer patients.

### Conflicts of interest

The authors declare no conflict of interest.

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