Detection and clinical significance of DNA repair gene ERCC8 tag SNPs in gastric cancer

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Cite this article as: *Lu X, Chen F, Liu X, et al. Detection and clinical significance of DNA repair gene ERCC8 tag SNPs in gastric cancer. Turk J Gastroenterol 2018; 29: 392-6.*

ABSTRACT

Background/Aims: *Excision repair cross-complementing group 8 (ERCC8) is one of the members of the nucleotide excision repair pathway. This study aimed to explore the association between ERCC8 tag single nucleotide polymorphisms (SNPs) and gastric cancer.*

Materials and Methods: *Totally, 120 patients with gastric cancer treated from March 2010 to March 2011 were selected as the observation group and 120 healthy individuals were selected as the control group during the same period. The Sequenom MassARRAY system was used to identify genotypes in these samples. The genetic locus of ERCC8 tag SNPs and the relevance of gastric cancer risk to the different ERCC8 genotypes alone or in combination with* Helicobacter pylori *infection were observed and analyzed. The AA, GA, and GG genotypes on rs158572 and rs158916 in the observation and control groups were compared.*

Results: *The results showed that the odds ratio of the different ERCC8 rs158572 and rs158916 genotypes was not significantly increased in the observation group compared with that in the control group. By contrast, in patients with* H. pylori *infection, the ERCC8 rs158572 GA/GG and rs158916 TT genotypes showed a 7.921-fold and 8.021-fold [95% confidence interval (CI)=4.022-15.921, p=0.029 and 95% CI=3.021-15.092, p=0.021, respectively] increased risk of gastric cancer than the AA and CT/CC genotypes, respectively.*

Conclusion: Helicobacter pylori *infection combined with ERCC8 rs158572 and rs158916 can be used as a predictive index of gastric cancer occurrence.*

Keywords: *DNA repair gene, ERCC8, gastric cancer, tag SNPs*

INTRODUCTION

Gastric cancer is the fourth most common malignant cancer worldwide. Although there has been remarkable improvement in cancer screening and treatment, the incidence of and mortality rate due to gastric cancer are still high (1). The occurrence of gastric cancer is related to dietary habits, living environment, and *Helicobacter pylori* infection (2-4). Genetic factors are also important, but their effect has not been well understood.

Genetic polymorphisms can cause hereditary and genetic susceptibility to a disease (6). Specifically, single nucleotide polymorphism (SNP) is the most common form of polymorphism and is thus often used to study genetic effects on human diseases. One or more SNPs can represent the SNPs of a monomer area, which is called a tag SNP (7,8). Deoxyribonucleic acid (DNA) repair systems play an important role in maintaining the integrity and stability of DNA (9,10) through processes including base excision repair, nucleotide excision repair (NER), mismatch repair, single-strand break repair, and double-strand break repair (11,12). When DNA is damaged, it activates its self-repair ability. However, the DNA repair ability significantly varies among individuals. Previous studies have shown that the sequences of the DNA repair gene can affect the repair ability of DNA (11,12). The polymorphisms of multiple genes involved in NER pathways are associated with different prognostics of gastric cancer; for example, excision repair cross-complementing group 2 (*ERCC2*) and excision repair cross-complementing group 6 (*ERCC6*) are associated with poor prognostic, while *ERCC1*, *ERCC5,* and *DDB2* predict longer overall survival (13). However, excision repair cross-complementing group 8 (*ERCC8*) is the core gene in the transcription-coupled repair pathway of NER; its genetic polymorphism in cancer occurrence and prognosis is unclear, and it needs further investigation for the results in the previous reports are not consistent (13-18).

Thus, in this study, we aimed to investigate the significance of ERCC8 tag SNPs and their combination with *H. pylori* infection in diagnosing gastric cancer.

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Received: ***October 11, 2017*** *Accepted:* ***January 29, 2018*** *Available online date:* ***May 30, 2018***

DOI: ***10.5152/tjg.2018.17662***

MATERIALS AND METHODS

Ethics

This study was conducted in accordance with the principles of the Declaration of Helsinki, and it was approved by the ethics committee of our hospital. Verbal informed consent was obtained from each subject.

Study subjects and materials

A total of 120 patients with gastric cancer treated at our hospital between March 2010 and March 2011 were selected as the observation group (mean age: 51.1±2.1 years), and 120 healthy individuals (mean age 50.9±2.0 years) were selected as the control group during the same period. All subjects were of the same race. Patients in the observation group were diagnosed with early gastric cancer by gastroscopy and a histopathologic examination.

Fasting venous blood from the included patients was collected, and blood serum was separated. The blood clot was preserved at -20°C. All DNA primers were purchased from Thermo Fisher Scientific Inc.; Waltham, MA, USA. SNP-type tests were performed using the iPLEX Gold Reagent Kit purchased from Sequenom Inc. The Sequenom MassARRAY high-throughput technology platform was used for genotype detection. The phenol-chloroform extraction method was used to extract genomic DNA.

Tag SNP selection

Haploview software was used to screen *ERCC8* tag SNPs, and then, FastSNP was used to evaluate the function and risk of candidate tag SNPs to select an SNP with a potential effect of clinical interest. The risk evaluation of tag SNPs was conducted according to the genetic risk assessment program. After screening, two potential tag SNPs (rs158572 and rs158916) were selected for further study.

SNP genotyping

A 500 µL blood clot was put in a centrifuge tube, and then, 800 µL TE was added. After they were mixed evenly, DNA was extracted. The sample was centrifuged at 10000 rpm for 5 min. Subsequently, 400 µL LTE, 25 µL 10% SDS, and 5 µL of 20mg/mL PK solution were sequentially added to the sample that was digested overnight. On the next day, the supernatant was discarded, and the same volume of phenol was added. After whirling for 15 min and centrifugation, the supernatant was discarded. Then, a 1:1 mixture of chloroform:phenol was added, the sample was centrifuged, and the supernatant was discarded again, after which two times of absolute ethyl alcohol and 3 M sodium acetate were added. Finally, the sample was used in the precipitation reaction at -20°C, after which the supernatant was discarded, and 75% ethyl alcohol was added for centrifuge. The precipitation substance was dried and preserved after discarding the supernatant from the sample.

The primer sequences of candidate SNPs were designed, and polymerase chain reaction (PCR) was performed. Then, the PCR product was purified, and single-base extension was performed.

Statistical analysis

Statistical Package for Social Sciences (SPSS) version 17.0 (SPSS Inc.; Chicago, IL, USA) was used to analyze the data. The chi-squared test was performed to compare the difference between the genes in the two groups, and the confidence interval was 95%.

RESULTS

ERCC8 tag SNPs and the risk of gastric cancer

The expression of AA, GA, and GG on rs158572 and of TT, CT, and CC on rs158916 in the observation and control group, respectively, were compared. Compared with the AA genotype, the rs158572 GA genotype, GG genotype, and GA/GG genotype were not associated with an increased risk of gastric cancer ($p>0.05$, Table 1). Moreover, compared with the rs158916 TT genotype, no positive relationship was observed between the CT genotype, CC genotype, CT/CC genotype, and an increased risk of gastric cancer ($p>0.05$, Table 2). It has been indicated that the ERCC8 tag SNPs increased the risk of gastric cancer.

ERCC8 tag SNPs combined with H. pylori infection and the risk of gastric cancer

As *H. pylori* infection is one of the main factors that causes gastric cancer, *H. pylori* infection in patients was observed and the relevance of the expression of AA, GA, and GG on rs158572 and of TT, CT, and CC on rs158916 was analyzed; the risk of gastric cancer in the subpopulations with regard to *H. pylori* infection was evaluated. As shown in Table 3, the risk of gastric cancer in patients carrying ERCC8 rs158572 and rs158916 significantly increased, regardless of whether it was combined with *H. pylori* infection ($p<0.05$). Moreover, compared with the AA genotype, the risk of gastric cancer in patients with the rs158572 GA/GG genotype combined with *H. pylori* infection was much higher than that in patients without *H. pylori* infection (OR=7.921, 95% CI=4.022-15.921, p=0.029 and OR=1.122, 95% CI=0.709-2.022, p=0.012,

Table 1. The occurrence rate of gastric cancer related to the ERCC8 tag SNP rs158572 in the observation and control groups

Group	Observation group	Control group	OR (95% CI)	p
AA	102	109	1.0	
GA	45	34	1.211 (0.802-1.722)	0.309
GG	9	3	4.099 (0.421-34.22)	0.212
GA/GG	54	37	1.402 (0.872-2.012)	0.22

Table 2. The occurrence rate of gastric cancer related to the ERCC8 tag SNP rs158916 in the observation and control groups

Group	Observation group	Control group	OR (95% CI)	p
TT	98	102	1.0	
CT	29	35	0.810 (0.563-1.121)	0.200
CC	4	4	0.850 (0.312-2.312)	0.792
CT/CC	33	39	0.821 (0.598-1.312)	0.221

Table 2. The occurrence rate of gastric cancer related to the ERCC8 tag SNP rs158916 in the observation and control groups

Group		HP (-)	HP (+)
rs158572	Control/patient		
AA		75/27	34/75
GA/GG		28/12	11/34
OR (95% CI)		1.122 (0.709–2.022)	7.921 (4.022-15.921)
p		0.012	0.029
rs158916	Control/patient		
CT/CC		90/22	30/98
TT		32/41	43/65
OR (95% CI)		1.221 (0.901-3.421)	8.021 (3.021-15.092)
p		0.031	0.021

respectively). Furthermore, compared with the TT genotype, the risk of gastric cancer for patients with the rs158916 CT/CC genotype combined with *H. pylori* infection is much higher than that in patients without *H. pylori* infection (OR=8.021, 95% CI=3.021–15.092, p=0.021 and OR=1.221, 95% CI=0.901-3.421, p=0.031, respectively).

DISCUSSION

The morbidity rate due to gastric cancer is the fourth highest worldwide among all cancers, whereas the mortality rate due to the same holds the second place among all cancers worldwide (19). The modality of gastric cancer is related not only to environment and dietary habits but also to gene mutations in the human body (20,21). Many studies have shown that patients with gastric cancer do not have fresh vegetables and fruit in their daily diet; in addition, they often smoke or eat food with high salinity and high stimulus (22,23). Besides, long-term *H. pylori* infection is a risk factor of gastric cancer, but only a minority of patients with *H. pylori* infection will develop gastric cancer (19,24). In recent years, the occurrence rate of gastric cancer has been rising, and operative treatment is the main treatment method. Patients who receive early operative treatment have good prognosis, but there are usually distant metastases in patients at the advanced stage (25,26). Therefore, it is important to increase the detection rate of gastric cancer and understand the cancer condition for providing timely treatment. At present, individual prevention and gene treatment have been increasingly used for malignant cancers, which is a hot research topic (27,28). As a single gene mutation does not have a significant effect on cancer pathogenesis, predictive value is limited (29). The development of SNP technology is thus necessary for malignant cancers, particularly gastric cancer.

During the development of cancer, some genes are critical or even decisive, and such genes can combine with environmental factors to cause malignant cancer in the body. For instance, a carcinogen attacks normal cells in the human body and causes DNA damage. A normal body will activate the DNA repair system to automatically repair DNA, which is a complicated process and involves various enzymes and proteins (30,31). However, a potential mutation during the DNA repair may change DNA function and cause unlimited proliferation and uncontrolled differentiation. Specifically, the SNP can affect the enzyme and thus affect cancer susceptibility (32). *ERCC1* is an important member of nucleotide circumscribed repair enzymes, as well as a critical DNA repair gene that makes cells survive, as ERCC can recognize and excise damage. To date, there are few studies on *XRCC1* polymorphism and cancer susceptibility, but fewer are concerned with *ERCC8* (33).

In our study, the results showed that *H. pylori* infection combined with *ERCC8* gene expression had a positive correlation with gastric cancer. *H. pylori* infection is an important factor that causes gastric cancer, and *ERCC8* expression is correlated with gastric cancer. According to the results, the DNA repair gene *ERCC8* tag SNPs have clinical significance and practical value in detecting gastric cancer. More specifically, the AA, GA, and GG genotypes on rs158572 and rs158916 in the observation and control groups were compared. It was revealed that the gene locus on rs158572 in the observation and control groups had no statistical difference. Besides, the gene locus on rs158916 in the two groups had no statistical difference. In contrast, *H. pylori* infection had a certain correlation with the two tag SNPs. The risk of gastric cancer in patients carrying *ERCC8* rs158572 and rs158916 combined with *H. pylori* infection was significantly increased.

The above findings contribute to a better understanding of the role of genes in causing cancer, in the context that research in genetics has become a major trend in clinical medicine research. Research on the occurrence of malignant cancer induced by aberrant methylation-caused gene disability is a hotspot in the field of genetics (34,35). Individual treatment based on genes will be the main trend in the future, with the interpretation of human genetic maps and advances in genetic disease research.

The exploration of DNA repair gene *ERCC8* tag SNPs in patients with gastric cancer, as well as the effects of various factors on genetic susceptibility, has significant implications in the selection of a preventive detection indicator. This also has significance in the screening of the high-risk population with gastric cancer, e.g., patients with *H. pylori* infection, long-term alcoholism, poor dietary habits, and gastritis.

Although the results of our present study are promising, there are several limitations that have to be taken into consideration. First, a relatively small sample size in the present study may result in insufficient statistical power to detect the relationship between ERCC8 rs158572, rs158916, and gastric cancer. Second, because our research was designed as a clinical study, the possibility of selection bias is exited that it cannot represent the general public. Finally, because all subjects in our study are Chinese, the results of our study cannot be directly generalized to other ethnic groups.

In conclusion, *H. pylori* infection combined with ERCC8 rs158572 and rs158916 can be used as a predictive index of gastric cancer occurrence.

Ethics Committee Approval: Ethics Committee Approval has received for this study from the Ethics Committee of the People's Hospital of Wenshan Prefecture.

Informed Consent: Verbal informed consent was obtained from the patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author contributions: Concept - X.L.; Design - X.L.; Supervision - X.L., F.C., X.W.L., D.Y., Y.Z.; Fundings - X.L.; Materials - X.L., F.C.; Data Collection and/or Processing - F.C., X.W.L., D.Y., Y.Z., X.H., D.Z.; Analysis and/or Interpretation - X.L., F.C., X.W.L., D.Y., D.Z.; Literature Search - X.L., F.C., Y.Z.; Writing - X.L., F.C., X.W.L., X.H.; Critical Reviews - X.L.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: This work was supported by Basic Application Project of Wenshan Prefecture (Project No: 2060402; Application Research and Development).

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