Flap endonuclease-1 rs174538 G>A polymorphisms are associated with the risk of esophageal cancer in a Chinese population

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Esophageal cancer; FEN1; molecular epidemiology; polymorphism.

Abstract

Background: Esophageal cancer has a high mortality rate, particularly in Asia, and there are obvious racial differences in regard to incidence. The purpose of our study was to assess the genetic susceptibility of functional single nucleotide polymorphisms in flap endonuclease-1 (FEN1) in esophageal squamous cell carcinoma ESCC.

Methods: Clinical blood samples of 629 ESCC cases and 686 control samples were collected. The ligation detection reaction method was used to determine FEN1 rs174538 G>A genotypes.

Results: A significantly decreased risk of ESCC was associated with FEN1 rs174538 GA genotypes among patients under 63 years old.

Conclusions: Our results suggest that functional polymorphism FEN1 rs174538 G>A might affect personal susceptibility to ESCC. This result provides a solid theoretical foundation for further clinical study using larger sample sizes.

Introduction

Esophageal cancer occurs in the esophageal epithelium, and is accompanied by high rates of morbidity and mortality. Esophageal cancer can be divided into two pathological types: esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EADC). Although there are many treatment methods for esophageal cancer, including surgery, radiotherapy, and chemotherapy, these treatment methods have a poor effect, and most patients die within five years after treatment, with only 5–10% of patients surviving longer than five years. Esophageal cancer has a high incidence rate, accounting for 50% of the world’s cancer. China has one of the highest mortality rates of esophageal cancer; therefore, domestic scientists are focused upon the study of esophageal cancer.

Flap endonuclease-1 (FEN1) is a protein involved in DNA replication repair, located on human chromosome 11q12 ~ 13.1. It is involved in the lagging strand DNA synthesis, DNA base excision repair, the non-homologous end joining and homologous recombination process, and plays a vital role in maintaining genome stability. In addition, FEN1 is also involved in apoptosis and can effectively regulate apoptotic products, thus ensuring the smooth progress of apoptosis. Previous studies have shown that FEN1 is related to the development of autoimmune diseases, cancer, and other diseases.

Studies have revealed that a loss of RAD27 (homologue of human FEN-1) stimulates a variety of mutagenic and clastogenic events, including a significant increase in the rate of spontaneous mutation and enhanced sensitivity to DNA damage. Meanwhile, the mutant phenotype has been found in yeast cells, suggesting that the FEN1 mutant plays a potential role in mammalian genomic instability and tumorigenesis. Another study demonstrated that in a
mouse model, sporadic tumors, mainly identified as lung cancer, developed in 70% of mice carrying the E160D FEN1 mutation. A recent study showed that two single nucleotide polymorphisms (SNPs) of FEN1 genes (−69G>A and 4150G>T) were associated with the risk of lung cancer. However, a correlation with the risk of esophageal, liver, stomach, and colorectal cancers has not yet been established. From a molecular level, it is important to explore the molecular mechanisms of FEN1 functional genetic variants in ESCC in order to provide a theoretical basis for early diagnosis and to establish effective treatment programs.

We selected 629 patients with ESCC and 686 control samples without cancer to assess FEN1 rs174538 G>A SNP and ESCC risk. We found that the existence of FEN1 rs174538 G>A polymorphisms and susceptibility to ESCC was significantly correlated. Compared with the GG genotype, the GA genotype significantly reduces the risk of developing ESCC (GA vs. GG: adjusted odds ratio [OR] 0.81, 95% confidence interval [CI] 0.64–1.04; \( P = 0.092 \)). We performed stratification analyses by age, gender, smoking, and alcohol consumption, and the results showed that age had an effect on the relationship between the polymorphisms and susceptibility to ESCC.

When the FEN1 rs174538 GG homozygote genotype was used as the reference group, the GA genotype was associated with a borderline statistically significant decreased risk of ESCC (GA vs. GG: adjusted OR 0.81, 95% CI 0.64–1.04; \( P = 0.092 \)).

### Polymorphism genotyping

Blood was collected from each patient and transferred into ethylene-diamine-tetraacetic acid vacutainers. Genomic DNA isolation from whole blood was performed using the QIAamp DNA Blood Mini Kit (Qiagen, Berlin, Germany). The blood DNA was amplified by PCR according to the manufacturer’s protocol. The samples were genotyped using the ligation detection reaction method, as previously described.

### Statistical analyses

Differences in the distribution of demographic characteristics, selected variables, and genotypes of the FEN1 rs174538 G>A variant between the patients and controls were evaluated using Student’s \( t \) and \( \chi^2 \) tests. The connections between the FEN1 rs174538 SNP and risk of ESCC were examined by computing the ORs and 95% CIs using logistic regression analyses and adjusting for age, gender, smoking, and drinking status. All statistical analyses were performed using SAS 9.1.3 (SAS Institute, Cary, NC, USA).

### Results

#### Subject characteristics

Table 1 shows the basic information of the 629 ESCC patients and 686 controls. Age and gender were not significantly different between the case and control groups.

### Method

#### Study subjects

The Review Board of Jiangsu University (Zhenjiang, China) approved the study. All subjects provided written informed consent. The 629 patients were recruited from the Affiliated People’s Hospital of Jiangsu University and Affiliated Hospital of Jiangsu University (Zhenjiang, China) between October 2008 and June 2013. The 686 control subjects were selected based on physical examination and matched for age (±5 years) and gender to the ESCC patients during the same time period. Each subject was interviewed using a questionnaire to collect information on demographic characteristics, smoking, drinking, age, gender, and diet. Each subject donated 2 mL venous blood, which was used for coming assay and FEN1 genotyping. Subjects who smoked one cigarette per day for >1 year were defined as smokers, while subjects who consumed ≥3 alcoholic drinks a week for >6 months were considered alcohol drinkers.

### Table 1 Distribution of selected demographic variables and risk factors in ESCC cases and controls

| Variable          | Cases (n = 629) | Controls (n = 686) | \( P \)  \\
|-------------------|-----------------|-------------------|---------|
| Age (years)       |                 |                   |         |
| mean ± SD         | 62.85 (±8.13)   | 62.58 (±7.89)     | 0.541   |
| Age (years)       |                 |                   |         |
| <63               | 310             | 365               | 0.155   |
| ≥63               | 319             | 321               |         |
| Gender            |                 |                   |         |
| Male              | 444             | 461               | 0.185   |
| Female            | 185             | 225               |         |
| Tobacco use       |                 |                   | <0.001  |
| Never             | 355             | 499               |         |
| Ever              | 274             | 187               |         |
| Alcohol use       |                 |                   | <0.001  |
| Never             | 428             | 526               |         |
| Ever              | 201             | 160               |         |

†Two-sided \( \chi^2 \) and Student’s \( t \) tests; bold values are statistically significant (\( P < 0.05 \)); ESCC, esophageal squamous cell carcinoma; SD, standard deviation.
(P = 0.541 and P = 0.155), which indicates that these groups were adequately matched. However, there were significantly more smokers and drinkers in the case group (P < 0.001), suggesting that smoking and drinking are important factors leading to ESCC.

**Associations between flap endonuclease-1 (FEN1) rs174538 G>A polymorphisms and esophageal squamous cell carcinoma (ESCC) risk**

The genotype distributions of FEN1 rs174538 G>A in the cases and the controls are shown in Table 2. In the single locus analyses, the genotype frequencies of FEN1 rs174538 G>A were 40.33% (GG), 47.15% (GA), and 12.52% (AA) in the case patients and 36.60% (GG), 52.68% (GA), and 10.72% (AA) in the control subjects; the difference was not statistically significant (P = 0.138). In the recessive model, when the FEN1 rs174538 GG/AA genotypes were used as the reference group, neither the AA homozygote genotype (P = 0.138). In the recessive model, when the FEN1 rs174538 GG/AA genotypes were used as the reference group, neither the AA homozygote genotype nor the GA/AA homozygote genotypes were associated with a significantly lower ESCC risk (GG/AA vs. GA/AA: adjusted OR 0.70, 95% CI 0.50–0.97; P = 0.034, P< 0.001). In patients aged under 63 years, when the FEN1 rs174538 GG genotypes were used as the reference group, the FEN1 rs174538 GA/AA genotypes were associated with a significantly lower ESCC risk (GA/AA vs. GG: adjusted OR 0.70, 95% CI 0.50–0.97; P = 0.034, P< 0.001).

**Stratified analyses of association between FEN1 polymorphisms and ESCC risk**

To evaluate the effects of FEN1 rs174538 G>A genotypes on ESCC risk according to age, gender, smoking, and alcohol drinking status, we performed stratification analyses in a recessive model (Table 3). A significantly decreased risk of ESCC was associated with the FEN1 rs174538 GA genotypes among patients under 63 years old (GA vs. GG: adjusted OR 0.63, 95% CI 0.45–0.90; P = 0.010, P< 0.001). In patients aged under 63 years, when the FEN1 rs174538 GA/AA genotypes were used as the reference group, the FEN1 rs174538 GA/AA genotypes were associated with a significantly lower ESCC risk (GA/AA vs. GG: adjusted OR 0.70, 95% CI 0.50–0.97; P = 0.034, P< 0.001).

**Discussion**

We employed a gene-based approach in a case–control design to examine the association between SNPs in the FEN1 locus and the risk of developing ESCC. Our multi-level logistic analysis indicated that a significantly decreased risk of ESCC was associated with the FEN1 rs174538 GA genotypes in patients aged under 63 years.

The presence of FEN1, an essential nuclease, has been confirmed across different species, from archaeabacteria to human.18 FEN1 is an important tumor suppressor,7 and its function is regulated at the post-translational level, such as in acetylation,19 protein-protein interaction,20,21 and phosphorylation.22 Kucherlapati et al. reported that mice homozygous for FEN1 knockout have an embryonic lethal phenotype, but FEN1 heterozygous knockout mice appear to be normal.23 In recent years, a group of researchers have constructed a transgenic mouse model carrying the E160D FEN1mutation, which frequently occurs in cancer.31 As stated above, missing FEN1 leads to the mutator phenotype and apoptotic DNA fragment damage, resulting in genomic instability, chronic inflammation, and the initiation of cancer.12 The results of these reports demonstrate that FEN1 is a cancer susceptibility gene. Future studies

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**Table 2 Logistic regression analyses of associations between FEN1 rs174538 G > A polymorphism and risk of ESCC**

| Genotype | Cases (n = 629) | Controls (n = 686) | Crude OR (95% CI) | P | Adjusted OR† (95% CI) | P |
|----------|----------------|-------------------|------------------|---|---------------------|---|
| **FEN1 rs174538 G>A** | | | | | | |
| GG       | 248            | 239               | 1.00             | 1.00 |                       |   |
| GA       | 290            | 344               | 0.81 (0.64–1.03) | 0.085 | 0.81 (0.64–1.04) | 0.092 |
| AA       | 77             | 70                | 1.06 (0.73–1.53) | 0.757 | 1.05 (0.72–1.53) | 0.802 |
| **AA vs. GA vs. GG** | | | | | | 0.138 |
| GA + AA  | 367            | 414               | 0.85 (0.68–1.07) | 0.173 | 0.85 (0.68–1.07) | 0.176 |
| GG + GA  | 538            | 583               | 1.00             | 1.00 |                       |   |
| AA       | 77             | 70                | 1.19 (0.85–1.68) | 0.317 | 1.18 (0.83–1.68) | 0.355 |
| A allele | 444            | 484               | 1.00             | 1.00 |                       |   |

†Adjusted for age, gender, smoking, and drinking status. CI, confidence interval; ESCC, esophageal squamous cell carcinoma; FEN1, flap endonuclease-1; OR, odds ratio.
could determine whether SNPs in FEN1 may modify cancer risk by affecting FEN1 expression and function.

Accumulating evidence reveals that genetic polymorphisms in gene promoter and 3’-UTR regions may affect transcriptional and posttranscriptional expression.\(^{24}\) Recent research has shown that FEN1 rs174538 G and 4150 G alleles can significantly reduce FEN1 messenger RNA expression in normal gastrointestinal tissues, and have been associated with additional gastrointestinal cancer risks compared to FEN1 rs174538A and 4150T alleles.\(^{25}\) In our study, when the FEN1 rs174538 GG homozygote genotype was used as the reference group, the GA genotype was associated with a borderline statistically significantly decreased ESCC risk.

Previous studies have shown that the FEN1 rs174538G>A SNP located in the promoter region causes increased promoter activity. These findings indicate that naturally occurring genetic polymorphisms in the regulation regions of cancer-related genes may represent a significant potential factor for cancer risk.\(^{15,26}\) These results are also consistent with findings in hepatocellular carcinoma, and breast, lung, esophageal, gastric, and colorectal cancers in centers across China.\(^{15,25,27,28}\)

Our study has some limitations. We collected a limited number of cases and controls, which might not be a good representation because there was insufficient recurrence and survival information and no cases of tumor metastasis. Furthermore, the limited sample size also affected post-assessment of the role of polymorphism analysis in ESCC progression and prognosis.

In summary, our results suggest that the functional polymorphism FEN1 rs174538 G>A might affect personal susceptibility to ESCC. This result provides a solid theoretical foundation for future study to explore whether the existence of FEN1 genetic polymorphisms could be potentially useful for ESCC diagnosis.

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**Disclosure**

No authors report any conflict of interest.
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