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ORIGINAL ARTICLE

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

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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.[1]Esophageal cancer can be divided into two pathological types: esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EADC).[2,3] Although there are many treatment methods for esophageal cancer, including surgery, radiation therapy, 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.[4,5] 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.[6] it is involved in the lagging strand DNA synthesis,[7] DNA base excision repair,[8] the non-homologous end joining and homologous recombination process,[9,10] 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.[11] Previous studies have shown that FEN1 is related to the development of autoimmune diseases, cancer, and other diseases.[12]

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.[11,13,14] 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.[7]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.[12] 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.[15] 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 significantly decreased risk of ESCC (GA vs. GG: adjusted OR 0.81, 95% CI 0.64–1.04; $P = 0.092$).

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.

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).[16] 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.[17]

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 χ^2tests. 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

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

	Cases (n = 629)		Controls (n = 686)		
Variable	N	%	N	%	P†
Age (years)					0.541
mean ± SD	62.85 (±8.13)		62.58 (±7.89)		
Age (years)					0.155
<63	310	49.28	365	53.21	
≥63	319	50.72	321	46.79	
Gender					0.185
Male	444	70.59	461	67.20	
Female	185	29.41	225	32.80	
Tobacco use					<0.001
Never	355	56.44	499	72.74	
Ever	274	43.56	187	27.26	
Alcohol use					<0.001
Never	428	68.04	526	76.68	
Ever	201	31.96	160	23.32	

†Two-sided χ^2 and Student's t tests; bold values are statistically significant ($P < 0.05$). ESCC, esophageal squamous cell carcinoma; SD, standard deviation.

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

	Cases (n = 629)		Controls (n = 686)					
Genotype	N	%	N	%	Crude OR (95% CI)	*P*	Adjusted OR† (95% CI)	*P*
FEN1 rs174538 G>A								
GG	248	40.33	239	36.60	1.00		1.00	
GA	290	47.15	344	52.68	0.81 (0.64–1.03)	0.085	0.81 (0.64–1.04)	0.092
AA	77	12.52	70	10.72	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	59.67	414	63.40	0.85 (0.68–1.07)	0.173	0.85 (0.68–1.07)	0.176
GG + GA	538	87.48	583	89.28	1.00		1.00	
AA	77	12.52	70	10.72	1.19 (0.85–1.68)	0.317	1.18 (0.83–1.68)	0.355
A allele	444	36.10	484	37.06				

†Adjusted for age, gender, smoking, and drinking status. CI, confidence interval; ESCC, esophageal squamous cell carcinoma; FEN1, flap endonuclease-1; OR, odds ratio.

(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 (AA vs. GG/AA: adjusted OR 1.18, 95% CI 0.83–1.68; P = 0.355), nor the GA/AA homozygote genotype (GA/AA vs. GG/AA: adjusted OR 0.85, 95% CI 0.68–1.07; P = 0.176) were associated with a risk of ESCC. When the FEN1 rs174538 GG homozygote genotype was used as the reference group, the GA genotype was associated with a borderline statistically significantly decreased risk of ESCC (GA vs. GG: adjusted OR 0.81, 95% CI 0.64–1.04; P = 0.092), while the AA genotype was not associated with ESCC risk (AA vs. GG: adjusted OR 1.05, 95% CI 0.72–1.53; P = 0.802).

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_h = 0.027). 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_h = 0.045).

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 multilevel 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 archaebacteria 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.[11] 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

Table 3 Stratified analyses between *FEN1* rs174538 G>A polymorphism and ESCC risk by gender, age, smoking status, and alcohol consumption

	FEN1 rs174538 G>A (case/control)†				Adjusted OR‡ (95% CI); *P*; P_h§				
Variable	GG	GA	AA	GA + AA	GG	GA	AA	GA + AA	AA vs. (GA + GG)
Gender									
Male	180/160	203/236	51/42	254/278	1.00	0.76 (0.57–1.02); *P* 0.069; P_h 0.442	1.04 (0.65–1.68); *P* 0.865; P_h 0.687	0.81 (0.61–1.07); *P* 0.129; P_h 0.491	1.22 (0.78–1.90); *P* 0.390; P_h 0.743
Female	68/79	87/108	26/28	113/136	1.00	0.94 (0.61–1.45); *P* 0.780; P_h 0.442	1.05 (0.56–1.97); *P* 0.875; P_h 0.687	0.96 (0.64–1.45); *P* 0.861; P_h 0.491	1.09 (0.61–1.94); *P* 0.773; P_h 0.743
Age									
<63	126/112	138/196	37/33	175/229	1.00	**0.63 (0.45–0.90); *P* 0.010; P_h 0.027**	1.10 (0.63–1.92); *P* 0.731; P_h 0.747	**0.70 (0.50–0.97); *P* 0.034; P_h 0.045**	1.45 (0.86–2.43); *P* 0.163; P_h 0.590
≥63	122/127	152/148	40/37	192/185	1.00	1.06 (0.75–1.49); *P* 0.741; **P_h 0.027**	1.05 (0.62–1.77); *P* 0.854; P_h 0.747	1.06 (0.77–1.46); *P* 0.736; **P_h 0.045**	1.02 (0.63–1.65); *P* 0.943; P_h 0.590
Smoking status									
Never	139/169	161/251	44/55	205/306	1.00	0.78 (0.57–1.06); *P* 0.110; P_h 0.603	1.00 (0.63–1.58); *P* 0.990; P_h 0.371	0.82 (0.61–1.10); *P* 0.179; P_h 0.494	1.15 (0.75–1.76); *P* 0.529; P_h 0.444
Ever	109/70	129/93	33/15	162/108	1.00	0.82 (0.54–1.23); *P* 0.335; P_h 0.603	1.20 (0.59–2.42); *P* 0.615; P_h 0.371	0.87 (0.58–1.29); *P* 0.487; P_h 0.494	1.34 (0.69–2.61); *P* 0.383; P_h 0.444
Alcohol consumption									
Never	159/176	195/267	61/58	256/325	1.00	0.78 (0.58–1.04); *P* 0.089; P_h 0.773	1.05 (0.68–1.63); *P* 0.822; P_h 0.654	0.83 (0.62–1.09); *P* 0.179; P_h 0.961	1.22 (0.81–1.82); *P* 0.339; P_h 0.577
Ever	89/63	95/77	16/12	111/89	1.00	0.84 (0.53–1.32); *P* 0.452; P_h 0.773	1.06 (0.46–2.42); *P* 0.895; P_h 0.654	0.87 (0.56–1.35); *P* 0.529; P_h 0.961	1.16 (0.53–2.56); *P* 0.715; P_h 0.577

†The genotyping was successful in 615 (97.8%) esophageal squamous cell carcinoma (ESCC) cases, and 653 (95.2%) controls for flap endonuclease-1 (*FEN1*) rs174538 G>A. ‡Adjusted for age, gender, smoking status, and alcohol consumption (besides accordingly stratified factors) in a logistic regression model. §P_h for heterogeneity, bold values are statistically significant ($P < 0.05$). CI, confidence interval; 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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